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THE

Progressive Fish-Culturist

UNITED STATES DEPARTMENT OF THE INTERIOR
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BUREAU OF SPORT FISHERIES AND WILDLIFE

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, Secretary
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
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THE PROGRESSIVE FISH-CULTURIST

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A SCHEMATIC OUTLINE FOR THE PRESUMPTIVE IDENTIFICATION OF BACTERIAL DISEASES OF FISH

G. L. Bullock
Bureau of Sport Fisheries and Wildlife
Eastern Fish Disease Laboratory, Kearneysville, West Virginia

TO OBTAIN A POSITIVE DIAGNOSIS OF BACTERIAL DISEASES OF FISH, it is necessary to isolate and identify the causative bacteria. As the information concerning the isolation and identification of fish pathogens is scattered through many reports and papers, it is desirable to bring as much material as possible into a central outline. The purpose of the outline to be described here is to provide concise information and methods that will be of assistance to hatchery biologists or fish diagnosticians in the presumptive diagnosis of bacterial diseases of fish.

METHODS

The flow chart (see figure) is for the most part self-explanatory. In addition to the described procedures, only general comments are necessary.

In the gross examination of diseased fish (second entry on the flow chart), the questionnaire referred to is the one that has been prepared at this laboratory. A general instruction sheet accompanies the questionnaire. On this instruction sheet are listed proper methods of bacteriological examination, preservation for shipment, and other procedures used with the diseased fish. It is important that these instructions be followed closely in order that a complete and accurate diagnosis may be made. To obtain a copy, write to this laboratory.

The key to fish parasites is being prepared by Dr. G. L. Hoffman, also of this laboratory.

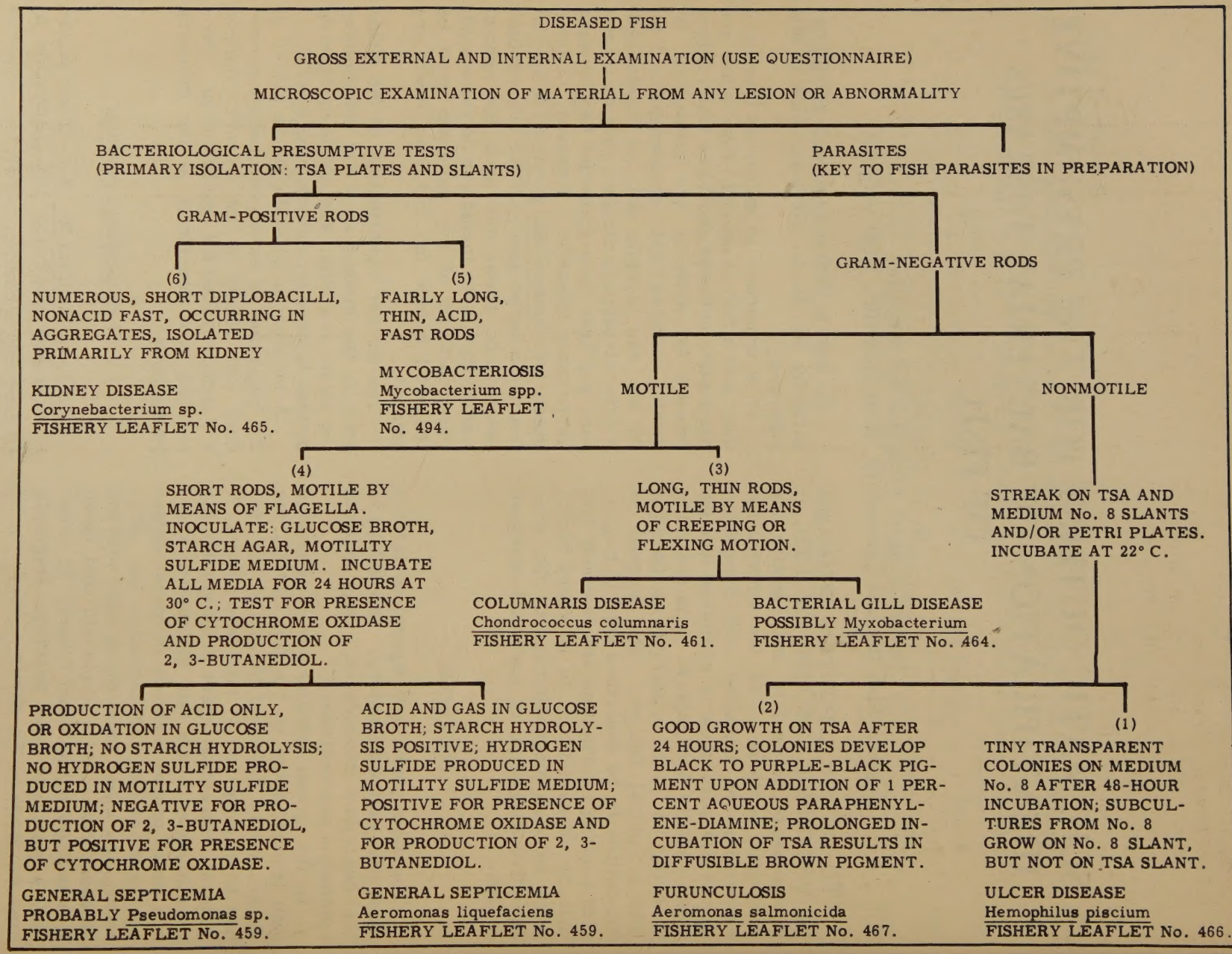
It is important to stress at this point that once a fish is opened, agar-slant and petri-plate cultures should be taken before smears for staining are prepared. By making cultures first, contamination from the air and utensils will be minimized. A general-purpose medium such as trypticase soy agar (see methods) can be used for primary isolation. However, in some cases (see Hemophilus piscium) a special medium must be used. If the microscopic examination shows the need for special media, then those media can be inoculated. Pure cultures of any pathogen must be prepared before the individual tests are started.

The Hucker modification of the Gram stain is the method used for determining the Gram reaction, and the hanging-drop method is used for determining motility.

A number in parentheses is placed at the beginning of each presumptive procedure and refers to the specific method and media needed for identification. Procedures are described in the following paragraphs.

(1) Hemophilus piscium

Leetown Medium No. 8.--This medium is normally used for the cultivation of Hemophilus piscium. Incubate cultures at 20° to 22° C.


DISEASED FISH
GROSS EXTERNAL AND INTERNAL EXAMINATION (USE QUESTIONNAIRE)
MICROSCOPIC EXAMINATION OF MATERIAL FROM ANY LESION OR ABNORMALITY
BACTERIOLOGICAL PRESUMPTIVE TESTS (PRIMARY ISOLATION: TSA PLATES AND SLANTS)
PARASITES (KEY TO FISH PARASITES IN PREPARATION)
GRAM-POSITIVE RODS
GRAM-NEGATIVE RODS
(6)
NUMEROUS, SHORT DIPLOBACILLI, NONACID FAST, OCCURRING IN AGGREGATES, ISOLATED PRIMARILY FROM KIDNEY
KIDNEY DISEASE
Corynebacterium sp.
FISHERY LEAFLET No. 465.
(5)
FAIRLY LONG, THIN, ACID, FAST RODS
MYCOBACTERIOSIS
Mycobacterium spp.
FISHERY LEAFLET No. 494.
MOTILE
NONMOTILE
(4)
SHORT RODS, MOTILE BY MEANS OF FLAGELLA. INOCULATE: GLUCOSE BROTH, STARCH AGAR, MOTILITY SULFIDE MEDIUM. INCUBATE ALL MEDIA FOR 24 HOURS AT 30° C.; TEST FOR PRESENCE OF CYTOCHROME OXIDASE AND PRODUCTION OF 2, 3-BUTANEDIOL.
(3)
LONG, THIN RODS, MOTILE BY MEANS OF CREEPING OR FLEXING MOTION.
COLUMNARIS DISEASE
Chondrococcus columnaris
FISHERY LEAFLET No. 461.
BACTERIAL GILL DISEASE
POSSIBLY Myxobacterium
FISHERY LEAFLET No. 464.
STREAK ON TSA AND MEDIUM No. 8 SLANTS AND/OR PETRI PLATES. INCUBATE AT 22° C.
PRODUCTION OF ACID ONLY, OR OXIDATION IN GLUCOSE BROTH; NO STARCH HYDROLYSIS; NO HYDROGEN SULFIDE PRODUCED IN MOTILITY SULFIDE MEDIUM; NEGATIVE FOR PRODUCTION OF 2, 3-BUTANEDIOL, BUT POSITIVE FOR PRESENCE OF CYTOCHROME OXIDASE.
GENERAL SEPTICEMIA
PROBABLY Pseudomonas sp.
FISHERY LEAFLET No. 459.
ACID AND GAS IN GLUCOSE BROTH; STARCH HYDROLYSIS POSITIVE; HYDROGEN SULFIDE PRODUCED IN MOTILITY SULFIDE MEDIUM; POSITIVE FOR PRESENCE OF CYTOCHROME OXIDASE AND FOR PRODUCTION OF 2, 3-BUTANEDIOL.
GENERAL SEPTICEMIA
Aeromonas liquefaciens
FISHERY LEAFLET No. 459.
(2)
GOOD GROWTH ON TSA AFTER 24 HOURS; COLONIES DEVELOP BLACK TO PURPLE-BLACK PIGMENT UPON ADDITION OF 1 PERCENT AQUEOUS PARAPHENYLENE-DIAMINE; PROLONGED INCUBATION OF TSA RESULTS IN DIFFUSIBLE BROWN PIGMENT.
FURUNCULOSIS
Aeromonas salmonicida
FISHERY LEAFLET No. 467.
(1)
TINY TRANSPARENT COLONIES ON MEDIUM No. 8 AFTER 48-HOUR INCUBATION; SUBCULTURES FROM No. 8 GROW ON No. 8 SLANT, BUT NOT ON TSA SLANT.
ULCER DISEASE
Hemophilus piscium
FISHERY LEAFLET No. 466.

Proteose peptone No. 3 or 4	grams	5.0
Tryptic digest of casein (N-Z-Case,[1] Trypticase, or Bacto[2]-Casitone)	do.	5.0
Yeast extract	do.	5.0
Sodium chloride	do.	5.0
Maltose (technical)	do.	5.0
Agar	do.	15.0
Distilled water	liter	1.0
pH		about 6.8

After mixing all the ingredients, heat the flask in boiling water to dissolve the agar, distribute the medium in 10-milliliter amounts in test tubes, and sterilize for 15 minutes at 15 pounds of pressure. Before using, melt the agar, cool to 50° C., and aseptically add 0.5 milliliter of filter-sterilized fish peptone (Snieszko, Griffin, and Friddle, 1950) or 1 milliliter of a 2.0-milligram percent filter-sterilized solution of cocarboxylase (Griffin 1952) per 10 milliliters of medium. It is important not to reheat the medium after adding either fish peptone or cocarboxylase, because temperatures above 55° C. will inactivate these compounds. As this medium is infrequently used, a small number of tubes of the complete medium can be obtained by writing to this laboratory. The medium can then be stored until needed.

(2) Aeromonas salmonicida

Trypticase Soy Agar (TSA).--This medium is commercially available.[3] It is one of the media that will show the characteristic brown diffusible pigment produced by Aeromonas salmonicida. Prepare and sterilize as directed. Incubate cultures at 20° to 22° C.

One-Percent Aqueous Paraphenylenediamine.--This solution deteriorates rapidly at room temperature and must be prepared every 3 to 4 days. However, it will keep as long as 1 month under refrigeration.

(3) Chondrococcus columnaris and Bacterial Gill Disease

Chondrococcus columnaris is usually grown on Ordal's medium and incubated at 22° C.

Tryptone	grams	0.5
Yeast extract	do.	.5
Beef extract	do.	.2
Sodium acetate	do.	.2
Agar	do.	9.0
Distilled water	liter	1.0
pH		7.2 to 7.3

Material from the lesion or growth from the above medium, when suspended in water on a hanging-drop preparation, should show long, thin, flexible rods tending to form short columnlike masses.

Bacterial gill disease is thought to be caused by a Myxobacterium, although it has not been experimentally transmitted. Organisms that resemble Chondrococcus columnaris, therefore, may be isolated. Refer to Fishery Leaflet No. 464 for more information.

(4) Aeromonas liquefaciens and Pseudomonas spp.

The methods to be described for the identification of the above organisms have just been evaluated and tested on laboratory cultures. It is believed that these methods will prove their worth under field conditions. All media for the identification of these organisms should be incubated at 30° C. for 24 hours.

Glucose Broth[4].--Nutrient broth containing 5 milliliters of chlorphenol red (0.4 percent chlorphenol red in 25 percent ethyl alcohol) per liter is used as the basal medium.

Distribute 9-milliliter amounts of the medium in test tubes, and insert inverted fermentation tubes (10 by 60 millimeters).

[1] Sheffield Chemical Company, Inc., Norwich, New York.

[2] Difco Laboratories, Detroit, Michigan.

[3] Baltimore Biological Laboratory, Baltimore, Maryland.

[4] Glucose synonymous with dextrose.

After sterilizing the medium for 15 minutes at 15 pounds of pressure, aseptically add to each tube of medium 1 milliliter of a 10-percent glucose solution (sterilized for 15 minutes at 15 pounds of pressure). Because sterilization of the glucose and basal medium together may cause chemical changes in the glucose, it is better to sterilize the sugar separately and add it aseptically to the basal medium. Observe for acid and/or gas production or oxidation of glucose after the specified incubation period. The appearance of an acid reaction only at the top of the medium is usually indicative of oxidation of the glucose.

Motility Sulfide Medium (Bacto).--Prepare as directed, distribute 10-milliliter amounts in test tubes, and sterilize for 15 minutes at 15 pounds of pressure. Inoculate by stabbing the medium from top to bottom, using a bacteriological needle. Blackening of the medium after incubation indicates production of hydrogen sulfide.

Starch Hydrolysis.--Add 0.2 percent soluble starch to the nutrient agar before sterilization. Distribute the medium in 10-milliliter amounts and autoclave for 15 minutes at 15 pounds of pressure. After sterilization, slant the tubes and allow the medium to harden. Inoculate each slant by drawing a loop containing the inoculum up the middle of each tube of the medium. After incubation, test for starch hydrolysis by pipetting 1 milliliter of iodine solution (same as for Gram's stain) into each tube. Scrape off growth with a loop to allow penetration of the iodine over the growth area. The appearance of a deep blue color throughout the entire surface of the medium is negative for starch hydrolysis, whereas no color or slight blue color around the fringes of the medium is considered positive.

Cytochrome Oxidase.--The method that was employed by Gaby and Hadley (1957) and modified by Ewing and Johnson (1960) is used.

Inoculate nutrient agar slants as in starch hydrolysis. After incubation, add to each tube 2 to 3 drops of a 1-percent aqueous solution of para-aminodimethylaniline oxalate and 2 to 3 drops of 1 percent alpha-naphthol in 95 percent ethyl alcohol. Tilt the tubes in order to mix the reagents and allow them to flow over the growth area. The appearance of a deep blue in the growth area within 90 seconds is considered positive for the presence of cytochrome oxidase.

Production of 2, 3-Butanediol.--The test medium for the production of 2, 3-butanediol is as follows:

Nutrient broth	grams	8.0
Dibasic potassium phosphate	do.	5.0
Distilled water	liter	1.0

Distribute the medium in 4.5-milliliter amounts and sterilize for 15 minutes at 15 pounds of pressure. At the same time sterilize a 15-percent glucose solution. After both have cooled, add aseptically to each tube of basal medium 0.5 milliliter of the sterile glucose solution. After the specified incubation time, determine the production of 2, 3-butanediol, using the method of Neish (1952).

1. Pipette into each tube of fermentation broth 1.0 milliliter of 2.3 percent periodic acid[5/] (solution to be stored in brown glass-stoppered bottle).

2. Shake tubes and then allow them to stand undisturbed at room temperature for half an hour.

3. Add to each tube 1.5 milliliters of piperazine solution[6/] and 0.5 milliliter of 4.0 percent sodium nitroprusside solution (prepared daily).

Development of a distinct blue within 2 minutes is considered positive for the production of 2, 3-butanediol. Pale green is considered negative.

(5) Mycobacterium spp.

Acid-Fast Staining.--Prepare smears by crushing a small amount of material between two slides. Allow to dry and then

5/ G. Frederick Smith Chemical Company, Columbus, Ohio.

6/ Dissolve 25 grams of piperazine hexahydrate in 99 milliliters of distilled water. Add 1.3 milliliters of 87 percent formic acid, or its equivalent.

fix with methyl alcohol. Flood slides with concentrated carbol fuchsin and heat stain until it just begins to steam. Stain for 3 to 5 minutes with gentle heating. Rinse with distilled water and then decolorize with acid alcohol (3 percent concentrated hydrochloric acid in 95 percent ethyl alcohol) until just a suggestion of pink remains. Counterstain with methylene blue. Acid-fast cells will be red, while background and non-acid-fast cells will be blue. If desired, Mycobacterium can be cultured on TSA or Dorset Egg medium. However, it is a slow-growing organism, and sometimes isolation is difficult.

(6) Kidney Disease

Although it is not essential for diagnosis, the kidney-disease organism can be cultured on Ordal's medium:

Tryptose	grams	10.0
Beef extract	do.	3.0
Sodium chloride	do.	5.0
Yeast extract	do.	.5
L-cysteine hydrochloride	do.	1.0
Human blood, by volume	milliliters	200.0
Distilled water	to make 1.0 liter	

ADDITIONAL INFORMATION

Each presumptive method outlined on the flow chart is followed by the number of a pertinent fishery leaflet. The fishery leaflets give more detailed information on symptoms, treatment, etc., of particular diseases and should be referred to after presumptive identification is made. They may be obtained from the Fish and Wildlife Service, U. S. Department of Interior, Washington 25, D. C.

SUMMARY

A schematic outline, or flow chart, is presented to assist in the presumptive diagnosis of bacterial diseases of fish. Specific methods for the identification of each organism are described.

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1950. A new bacterium (Hemophilus piscium n. sp.) from ulcer disease of trout. Jour. Bact. 59 (6): 699-710.

It may soon be possible for the fish-culturist to hatch bass fry and stock nursery ponds without the burden of moving fry from brood ponds. When six rubberized-hair mats (upholstering material) were placed in a vegetation-free pond with a silt-covered bottom and considerable gravel at each end, largemouth bass spawned on at least five of the mats. One nest of eggs was successfully hatched in a catfish-egg incubator.

A MIDSUMMER PLANT OF RAINBOW TROUT IN A STRATIFIED TENNESSEE IMPOUNDMENT

F. Phillip Sharpe
Tennessee Game and Fish Commission
Nashville, Tennessee

OUTSTANDING AMONG THE MANY ACHIEVEMENTS OF THE TENNESSEE GAME AND FISH COMMISSION in recent years have been its profile studies on some of the large impoundments. As a direct result of the investigations it has been determined that certain of these bodies of water are capable of supporting rainbow trout (Salmo gairdneri) populations in the vast, well-oxygenated thermoclines that lie just below the surface. Preliminary investigations have revealed that there is no direct space competition between trout and the warm-water species of fish already present in the lakes.

During 1959 two such impoundments were stocked with token plants of rainbow trout fingerlings. The problem encountered so far in this particular section of the country has been the availability of suitable rainbow trout at the optimum time of stocking in relation to the surface temperature of the water. During the summer the high surface temperature of the water is considered unfavorable for conventional stocking, and until now no stocking had been attempted during this period. In this study skin-diving equipment was used to make a summer plant of rainbow trout and to observe the reaction of the trout as they encountered a new and entirely different environment. Important in any method of trout stocking is a knowledge of the initial behavior pattern of the trout when confronted with a new habitat, as well as an evaluation of the significance of their reaction. With this in mind, an experimental plant of rainbow trout in a thermocline was undertaken in an effort to make observations and to derive a method of stocking trout in stratified water at the most opportune time.

Design of Experiment and Area of Study

The thermocline stocking experiment was conducted on a small impoundment in north-central Tennessee that has stratification characteristics similar to those of the larger impoundments mentioned earlier. Several methods of stocking trout in the thermocline were discussed prior to the actual stocking; however, the method used was determined by the fact that it was desirable to observe the fish during and immediately after the stocking. To transport the fish to the thermocline, the author and a fellow worker used skin-diving equipment and large plastic bags. The basic goal was to introduce trout to the cooler waters of the thermocline without subjecting them to the rigors of passing through the hot surface water, and actually to acquaint the raceway-reared trout with the colder water in the lake.

Standing Stone Lake, located in the north-central section of the Cumberland Mountains in Tennessee, was chosen for the study. The lake contains approximately 80 surface acres of water and is

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Mr. Sharpe is now employed by the Bureau of Sport Fisheries and Wildlife, Branch of Fish Hatcheries, Atlanta, Georgia.

FIGURE 1.--A bag of rainbow trout is handed from the anchored boat to a diver.

1,300 feet above sea level. Maximum depth of the lake is 26 feet. Most of the watershed is inside the boundaries of Standing Stone State Park and is heavily forested. The lake is extremely clear at times, and it stratifies with the upper portion of the thermocline at the 10- to 12-foot level. At the time of the experiment the water temperature was 69° F. at the top of the thermocline and the layer of stratification was quite visible.

Water Analysis

Prior to the study, temperatures, oxygen and free carbon dioxide samples, and Secchi-disc readings were taken. Temperatures ranged from 83° at the surface to 54° F. at the maximum depth (26 feet). A temperature of 69° was recorded at the 11-foot level on 3 sample days before the experiment. Dissolved-oxygen content on 3 sample days ranged from 8.0 p.p.m. at the surface to 5.6 p.p.m. at the bottom. Free carbon dioxide was recorded at less than 5 p.p.m. at all depths. Secchi-disc readings, before and during the experiment, were at 13 feet. For 2 weeks before the experiment, very little moisture was recorded in the immediate area.

Equipment and Fish

Standard scuba gear, aqua-lung and regulator, face mask, and swim fins were used to transport the fish below the surface. About 25 yards from the unloading dock, a boat was anchored and used as a departure point for the divers. A marked line from the boat was anchored to the bottom and used for a descent guide and release marker.

Five hundred 6- to 8-inch rainbow trout were transported 126 miles from Flintville State Fish Hatchery to the lake in an aerated tank. The temperature of the tank water at the time of arrival at the lake was 61°. Lake water was added to the tank to temper the trout, allowing the temperature to rise slowly to the desired level.

After the trout had been adequately tempered, they were put in plastic bags containing about 3 gallons of water taken from the tank truck. Twenty to twenty-five fish were put in each bag. The bags of fish were quickly taken to the anchor boat, from which they were handed to a diver already in the water (figure 1).

Procedure

After the diver received the bag of fish, he allowed it to float on the surface of the

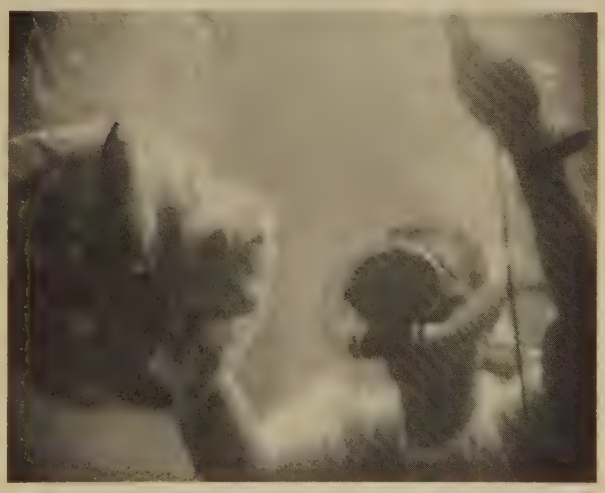


FIGURE 2.--Rainbow trout as they passed the 6-foot level in the lake.

water and forced the excess of air from the bag. Then he followed the marked line to a depth of 12 to 15 feet. Here the diver opened the bag and allowed the fish to swim free. The time elapsing from the removal of the fish from the tank truck until their release at the 12-foot level was approximately 5 minutes. A relatively slow descent was made, allowing the fish to adjust to the slight change in pressure encountered.

Surface Observations

Before the first descent with trout was attempted, 25 fish were released on the surface of the water and observed as long as possible. Trout released on the surface appeared to be severely affected by the high water temperature; they were immediately in distress. There was only a slight indication that the fish went to any depth immediately; rather, they went under the water for a short distance and then resurfaced. Many of the fish ran into the bank, and several died. It was impossible to follow the trout--other than those that became confused with the bank--for any length of time. It was evident that the trout were in severe distress when stocked in the warm surface water.

FIGURE 3.--Rainbow trout as they were released in the thermocline of the lake.

FIGURE 4.--Circular swimming pattern of rainbow trout released in the lake.

Underwater Observations

During the descent, the trout showed signs of confusion upon approaching the 6-foot level and all turned toward the surface (figure 2). As all the trout had been reared in shallow raceways, this reaction is believed to have been primarily a reaction to the loss of light, and not due to the slight pressure barrier encountered at this depth. After passing the 6-foot level, the trout became quiet and less mobile.

Upon release, the fish appeared more confused than distressed. Some of the fish swam readily from the bag, while others had to be forced out by turning the bag inside out (figure 3). After their release the fish righted themselves and began swimming in slow circles around the diver. Many times they bumped into the face mask of the diver and swam off again. Very close observation could be made, and a single trout could be followed for several minutes. No adverse effects were noted during this observation period.

The general pattern for each release was for the trout to float or swim from the bag, right themselves, and begin to swim in slow circles, rising and increasing the diameter of the circle (figure 4). Practically all fish returned to the 6- to 8-foot level, where the water temperature was 74° F. Here they continued the circular pattern of swimming and slowly worked back into the cooler water of the thermocline; eventually they disappeared. In each release several of the trout came to the surface but almost immediately returned to cooler water.

Observations After Stocking

One hour after stocking, the two biologist skindivers and one biologist in a boat covered the area in which the trout had been stocked. No trout were located on the surface or around the shoreline. A general survey of the lake the next day revealed that one trout had been washed over the spillway and was found dead on the riffles below the dam. Observations to date indicate that this stocking was entirely successful.

When this experiment was first designed, it was for the purpose of introducing trout to the thermocline and having them remain without any inclination to leave the colder water. Observation of the actions of the fish during and after stocking, however, made it apparent that this was not the effect. The main derivation from this study was the revelation of the thermocline to the fish. Even though the trout moved out of the thermocline a short distance, they were acquainted with the sensation of the presence of cold water and, as a result, were able to return to the lower depths after a short time. Fish stocked on the surface had no indication that the colder water lay a few feet below the surface. When fish stocked on the surface did reach the thermocline, they probably did so by chance during an effort to escape the warmer water. The reaction of the trout during the first few feet of descent is believed to be directly correlated with the loss of light, not with the slight difference in pressure at this shallow depth. The confused state of the fish immediately upon release was probably related to the crowding in the small plastic bag and the first encounter with a great loss of light. The absence of raceway walls and bottom to guide them undoubtedly had some effect on immediate stabilization of the trout. The fish adapted to changes very readily, and these changes appeared to have no lasting effect on the fish.

This type of stocking definitely has merit but is limited by the small number of fish that may be transported to any depth at one time. Also, in larger impoundments the thermocline surface will probably be at depths of 20 to 35 feet; if so, a definite pressure barrier may exist. A better method of stocking--a method that would avoid the pressure problem, the limited number of fish transported, and the difference in temperature--could be developed. An example would be an aluminum pipe, 8 to 12 inches in diameter, and long enough to reach from the tank truck to the thermocline. A slip-off cover incorporated on the lower end of the pipe could be removed after the pipe was emersed, allowing thermocline water to come up and be available at the surface of the lake. Fish could then be funneled into the pipe, with the only way of escape being at its lower end--into the thermocline. This might be one practical method of thermocline stocking, and others could be mentioned. However, the purpose of this study was to observe, and other proposed methods are bypassed. Of value in any type of thermocline stocking would be at least one scuba diver, available where the fish emerge into the thermocline, assisting in the emergence and observing the general reactions of the fish.

Acknowledgment

Appreciation is extended to David Murrian, photographer and experienced skindiver for the Tennessee Game and Fish Commission, and to the personnel of the Tennessee Game and Fish Commission who assisted in this study.

EARLY DEVELOPMENT OF THE CHANNEL CATFISH

Vishnu P. Saksena, University of Oklahoma, Norman, Oklahoma
Kiichiro Yamamoto, Faculty of Fisheries, Hokkaido University, Japan
and
Carl D. Riggs, University of Oklahoma, Norman, Oklahoma

MANY PAPERS DEALING WITH VARIOUS ASPECTS OF THE BIOLOGY OF THE CHANNEL CATFISH, Ictalurus punctatus, have been published (Riggs 1958), but the embryology of this species has not been thoroughly described. Murphree (1940) and Clemens and Sneed (1957) gave brief descriptions of the development, but they included no information about the early stages.

The development of two species of marine catfish, Bagre marinus (Merriman 1940), and Galichthys felis (Merriman 1940, Ward 1957), has been described. Although the observations on which these descriptions were based extended from the early to the later developmental stages of the embryo, they lacked continuity because continuous series of embryos were not collected.

This paper is a description of the gross morphology of the embryo of the channel catfish up to the development of the pectoral fin-buds. The eggs were obtained by K. E. Sneed and H. P. Clemens in June 1959 in the laboratory of the Tishomingo National Fish Hatchery, Oklahoma, from spawns which they induced by injecting A.P.L. (anterior pituitarylike hormone), using the method they described earlier (Sneed and Clemens 1959). The fertilized eggs were taken immediately from the 10-gallon aquarium in which they had been spawned and were put in another aquarium through which water flowed continuously. They were kept in this aquarium for 35 hours, during which time the temperature of the water ranged from 24.7° to 26.8° C. Beginning 50 minutes after spawning occurred, samples of the eggs were fixed in Smith's solution at 1-hour intervals for 34 hours. Each sample was kept in the fixative for 24 hours and then was washed for 12 to 18 hours in running water and preserved in 70 percent alcohol. To facilitate observations of the developing embryos, the chorion was removed after fixation and the eggs were stained with borax carmine for 5 hours. The diameter of the eggs fixed just after spawning, with the chorion removed, averaged 3.2 millimeters.

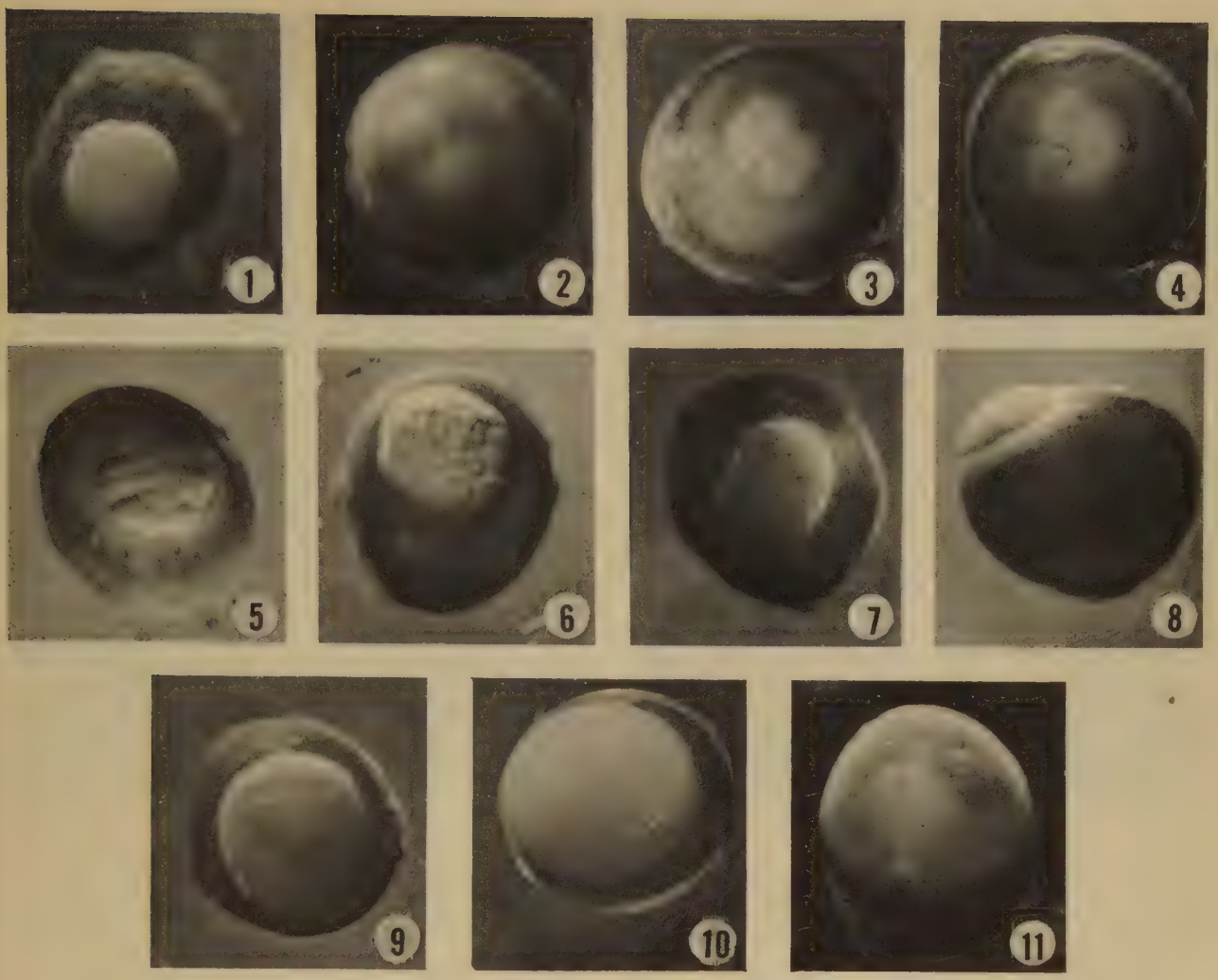
Observations

Eggs of channel catfish are demersal and adhesive. They adhere to each other in large masses, usually forming a single mass per spawn. Live, freshly laid eggs are light yellow; but soon after spawning the thick, tough chorion becomes dull and a large perivitelline space appears between the chorion and the egg.

The following descriptions are based on observations of the stained eggs with the chorion removed.

Button Stage (figure 1).--Zygotes preserved 50 minutes after spawning had a round, well-defined blastodisk, 1.3 millimeters in diameter, that was raised moderately from the yolk surface. There was a very thin extension of the protoplasm at the marginal region of the disk.

Two-Cell Stage (figure 2).--The first cleavage plane was median and vertical and divided the disk into two blastomeres

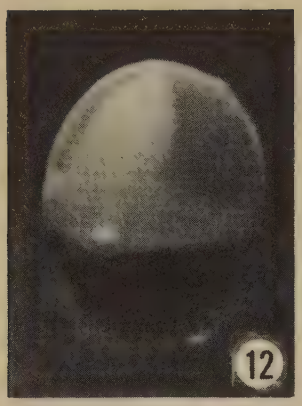


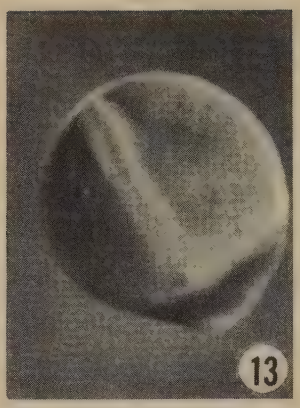
Explanation of figures

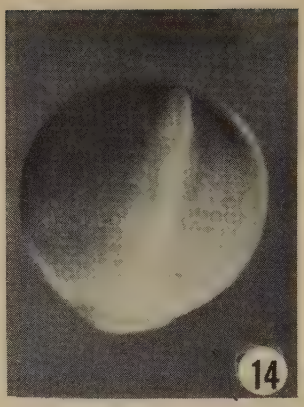
Gross appearance of blastoderm and embryo in fixed eggs after the removal of the chorion. The age is determined from time of spawning. Magnification, x 12.

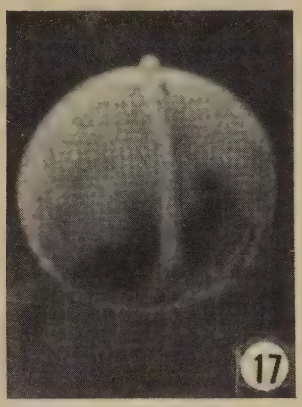
FIGURE 1.--Button stage. 50 minutes.
FIGURE 2.--Two-cell stage. 2.5 to 3.25 hours.
FIGURE 3.--Four-cell stage. 2.5 to 3.5 hours.
FIGURE 4.--Eight-cell stage. 3 to 4.5 hours.
FIGURE 5.--Sixteen-cell stage. 3.5 to 4.75 hours.
FIGURE 6.--Later segmentation stage. 5 hours.
FIGURE 7.--Later segmentation stage. 6 hours.
FIGURE 8.--Blastula stage. 8 hours.
FIGURE 9.--Early gastrula stage. 10 hours.
FIGURE 10.--Late gastrula stage. 11 hours.
FIGURE 11.--Blastoderm enveloping two-fifths of yolk sphere. 21 hours.

of almost equal size. The marginal extension of the protoplasm around the disk had become more clear than that of the button stage. Only 10 percent of the embryos preserved 3 hours 10 minutes after spawning were two-celled; the remainder were in more advanced stages. This, plus the fact that none of the zygotes examined 2 hours after spawning showed cleavage, seemed to indicate that the first cleavage









Explanation of figures

Gross appearance of blastoderm and embryo in fixed eggs after the removal of the chorion. The age is determined from time of spawning. Magnification, x 12.

FIGURE 12.--Blastoderm enveloping one-half of the yolk sphere. 23 hours.

FIGURE 13.--Blastoderm enveloping three-fourths of the yolk sphere. 25 hours.

FIGURE 14.--Blastoderm enveloping four-fifths of the yolk sphere. 26 hours.

FIGURE 15, 16.--Just before closing of the blastopore. 27 hours.

FIGURE 17, 18.--Optic-cup stage. 30 hours.

FIGURE 19.--Pectoral fin-bud stage. 34 hours.

occurred between 2.5 and 3 hours after spawning.

Four-Cell Stage (figure 3).--This stage was attained by 50 percent of the embryos preserved 3 hours 10 minutes after spawning. The plane of second cleavage was also vertical and was at right angles to the first; the four cells thus formed were similar in size and form.

Eight-Cell Stage (figure 4).--The third cleavage involved double, vertical planes which were parallel to the first plane. The blastoderm had elongated, and the resulting eight blastomeres were arranged in two rows of four cells each. The marginal protoplasm around the disk had become thick and clear, but apparently a true periblast had not yet appeared. Of the embryos preserved 3 hours 10 minutes and 4 hours 10 minutes after spawning, 40 and 25 percent, respectively, were in this stage.

Sixteen-Cell Stage (figure 5).--The two vertical planes of the fourth cleavage were parallel to the second cleavage plane. The blastoderm had become considerably raised from the yolk and had recovered its round shape. Fifty percent of the

embryos preserved 4 hours 10 minutes after spawning were in this stage.

Later Segmentation Stage (figures 6 and 7).--In this stage, composed of many small blastomeres, the blastoderm was rounded, compact, and projected above the yolk. A prominent periblast was marginal to the blastoderm. Embryos preserved from 5 to 7 hours after spawning were in this developmental stage.

Blastula Stage (figure 8).--Embryos preserved about 8 hours after spawning were typical blastulae. The blastoderm was somewhat flattened, and the individual blastomeres were now very small and could not be distinguished with the unaided eye. The marginal periblast had become quite distinct. The presence of the true blastocoele could not be ascertained without detailed observation after sectioning, and this was not done.

Early Gastrula Stage (figure 9).--About 10 hours after spawning, the initiation of gastrulation was indicated when the blastoderm, about 1.8 millimeters in diameter, became larger, more flattened, and began to expand over the yolk. Thickening of the rim of the blastoderm was still obscure.

Late Gastrula Stage (figure 10).--By 11 hours after spawning, the diameter of the blastoderm had increased to 2.4 millimeters, spreading over more yolk. Careful observations showed that the germ ring had already been formed as the thick rim of the blastoderm, and that the central area of the blastoderm had become thin. One portion of the germ ring had thickened more than the other part, indicating initial formation of the embryonic shield. In the median region of the shield, the caudal knob, projecting slightly over the rim of the blastoderm, was visible and marked the dorsal lip region. Most of the embryos preserved 16 hours after spawning were in this stage.

Blastoderm Enveloping Two-fifths of the Yolk Sphere (figure 11).--The blastoderm had overgrown about two-fifths of the yolk about 21 hours after spawning. The embryonic shield had grown forward from the dorsal lip region, and the caudal knob projected markedly over the rim of the blastoderm.

Blastoderm Enveloping One-half of the Yolk Sphere (figure 12).--About 23 hours after spawning, the embryonic shield had become elongated and raised conspicuously from the yolk surface with the median region thickened, indicating initial development of the neural tube and notochord. The caudal knob was prominent and projecting.

Blastoderm Enveloping Three-fourths of the Yolk Sphere (figure 13).--At about 25 hours, the blastoderm had grown over about three-fourths of the yolk, leaving a large yolk plug. The embryonic shield had been condensed to form a distinct keel. In the median line of the keel, a shallow furrow--the transitory neural groove--was present. The anterior end of the keel was enlarged, forming the anlage of the forebrain. The prominently projected caudal knob was recognizable in the posterior end of the keel.

Blastoderm Enveloping Four-fifths of the Yolk Sphere (figure 14).--In embryos at this stage (at 26 hours), the embryonic body, considerably raised from the yolk surface, could be distinguished from the lateral body, which was composed of a thin layer. The embryonic body had a clear forebrain with lateral, lobelike enlargements corresponding to the anlage of the optic vesicles. The transitory neural furrow still was visible along the surface of the median part of the embryonic body. Usually at this stage three to five somites were visible.

Just Before Closing of the Blastopore (figures 15 and 16).--About 27 hours after the spawning, the yolk had been almost entirely enveloped by the overgrowing germ ring. The opening of the blastopore was small, but a small yolk plug still remained. Dorsal, lateral, and ventral lips were readily discernible in the germ ring (figure 15). The form of the embryo was recognizably fishlike, with 10 to 11 somites, and the brain was beginning to divide into three general regions. The optic vesicles and the auditory placodes were visible (figure 16).

Optic-Cup Stage (figures 17 and 18).--About 30 hours after spawning, the blastopore had completely closed. The embryo,

which now had 15 to 16 somites, measured 3 millimeters. The forebrain, midbrain, and hindbrain were clearly differentiated; and the optic lobes had developed into the optic cups and lenses. The optic vesicles were also readily recognizable under the pericardial sac. The heart was a straight, simple tube. At the lower sides of the body, in front of the heart, piles of cells represented the beginnings of the pectoral fin buds; a distinct tail bud did not yet show. Kupfer's vesicle was situated in front of the caudal knob.

Pectoral Fin-Bud Stage (figure 19).--The embryo that was preserved 34 hours after spawning, 3.5 millimeters long and with about 24 somites, had well-developed globular pectoral fin buds readily visible with the unaided eye. The well-marked tail bud was elevated from the yolk surface. The lenses in the eyes were recognizable, but there were no melanophores. The heart on the floor of the pericardial sac was composed of two chambers. The otic vesicles were large and clear, but the presence of the otolith was not detectable from the outside. Kupfer's vesicle had almost disappeared at this stage.

Discussion

The features of the early embryonic development in the channel catfish resemble those of the chum salmon (Mahon and Hoar 1956) with respect to the relatively small blastodisk, the lack of a recognizable, true blastocoele, the early occurrence of a projected caudal knob, and a marked neural furrow.

Channel catfish eggs are about 3.2 millimeters in diameter without the chorion, smaller than those of the chum salmon (Mahon and Hoar 1956) and the Atlantic salmon (Battle 1944) and much smaller than those of the sea catfish, which measure 14 to 18 millimeters in diameter (Ward 1957). In the latter, at the presumed time of the closure of the blastopore, the optic vesicles, auditory placodes, and more than 10 somites are differentiated, but not the blood vessels, the gut, and the pronephros. Eggs and embryos of the channel catfish, developing more rapidly than those of the sea catfish, reached the four-cell stage by 3 hours and the blastula stage by 8 hours at 24.7° to 26.8° C.; at 29.5° C., the sea catfish took 7 hours to attain the four-cell stage and 20 hours to reach the blastula stage.

Summary

Eggs for the study of the early development of the channel catfish, Ictalurus punctatus, were obtained by means of hormone-induced spawning in aquaria.

The features of the development are characterized by a relatively small blastodisk, lack of a recognizable, true blastocoele, early occurrence of a projected caudal knob, and a marked neural furrow. The embryo differentiates the optic vesicles, the auditory placodes, and more than 10 somites by the time of the closure of the blastopore, about 30 hours after spawning. The pectoral fin-bud stage, about 3.5 millimeters long and of about 24 somites, was reached in 34 hours.

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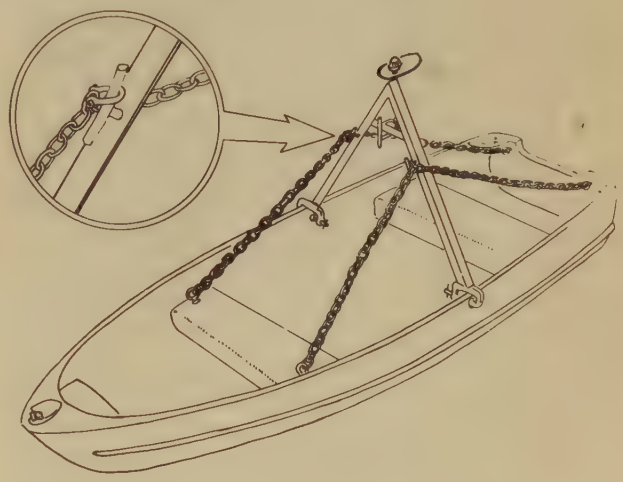
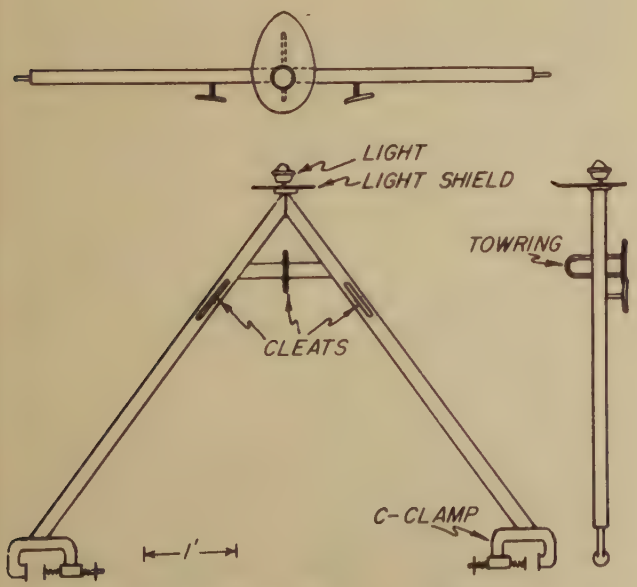
A FRAME USED AS A TOWING AID FOR SMALL BOATS

It is often difficult to tow plankton nets and other collecting gear from small boats because attaching the towline to the transom reduces maneuverability of the boat.

A device proved helpful on a 16-foot aluminum boat is a 3-foot A-frame (three pieces of 1.5-inch angle iron welded together) supported by stays. A 4-inch C-clamp is welded to the base of each leg for attaching the frame to the gunwales. A rope stay from the center cleat to the bow is adequate support, and a ring on the rear center of the crossbar is attachment point for the towline. For more rigid support, chain stays may be shackled fore and aft by eyebolts fitted to the hull. During towing, a shackle on each chain is put over the iron cleat welded to the forward face of each leg of the frame.





With the frame, meter plankton nets have been towed at speeds up to 2 knots, at the surface and on the bottom, in narrow, winding channels. Advantages of its use are: the boat is more maneuverable, the towline swings clear of the operator and motor, working space is not appreciably reduced, cost is low (materials and labor, $7.50), and the chains form guard-rails.

--ANTHONY L. PACHECO and GEORGE C. GRANT, Bureau of Commercial Fisheries, Millville, Delaware.

THE EFFECT OF GRADING ON THE TOTAL WEIGHT GAINED BY BROOK TROUT

Earl A. Pyle, Glen Hammer, and Arthur M. Phillips, Jr.
Bureau of Sport Fisheries and Wildlife
Cortland, New York

THREE REASONS are usually given for grading hatchery trout. First, grading makes possible the distribution of a uniform-sized trout, necessary if fish size is specified in a management program. Second, grading increases the efficiency of the hatchery management by reducing cannibalism in certain species of trout and permits more accurate estimates of weight by eliminating some of the variation in fish size in sample counts. Third, there is belief that smaller fish increase their growth rate after being removed from a mixed-size population, resulting in increased hatchery production. Brown (1946) presented data showing the existence of hierarchies in fish populations. She suggested "size hierarchy" as a possible cause of the retarded growth of the smaller brown trout. She pointed out that the removal of the larger fish accelerated the growth rate of the smaller fish, but when the larger fish were again placed with the smaller ones, their growth rate was again suppressed.

If fish of a specified distribution size are required, grading is necessary. If increased fish production is the objective of grading, the cost of grading should be justified by the amount of additional fish flesh produced. It appeared questionable that the growth retardation of dominated individuals of the hierarchies described by Brown was a significant factor in the total production of hatchery brook trout. With this in mind, experiments were designed to compare the weight gained by graded trout with that of ungraded populations.

Method

Eight 2,000-gram groups of brook trout were withdrawn from a single population and placed in standard hatchery troughs supplied with water averaging 51° F. in temperature (range, 48° to 65° F.) during the period. Four of these groups were graded into two sizes, and four were left undisturbed. The trout were graded by placing them in a rectangular box with grading bars at the bottom spaced one-fourth inch apart. The box with the fish was suspended at the water's surface for a 3-minute period without agitation of the fish or box. The fish that passed through the bars were placed in a separate trough and designated the small size; those remaining in the box were returned to the original trough and called the large size.

All troughs contained water 5 inches deep at a mid-point. Each trough of graded fish was equipped with a movable dam board. After the graded fish were weighed, the dam boards were placed so that the weight of fish per cubic foot of water was equal in both the graded and the ungraded troughs of fish.

The fish were weighed at 2-week intervals, and the dam boards were adjusted to maintain the equal weights of fish per cubic foot of water in the graded and ungraded groups of trout. The identity of the small and large fish of each graded

group was noted to allow a recombination of the weights for comparison with those of the ungraded groups.

By the end of 8 weeks, the weights of the ungraded fish had become too great for a single trough, and it was necessary to divide the fish between two troughs. The source of the fish in each trough was noted so that the weights could be recombined at a later date. Also at this time, the graded trout were regraded into three size groups by the previously described method. The smaller fish of the original small group were placed in one trough; the larger from the original small group and the smaller from the original large group were placed in a second trough; and the larger fish from the original large group were placed in a third. Thus, each originally graded group was divided into small, medium, and large fish. The source of each trough of graded fish was noted so that its weight could be compared later with the weights of the ungraded fish. The movable dam boards were adjusted in each trough to establish equal weights of fish per cubic foot of water in all experimental units.

The trout were fed the standard blower-type food of this hatchery. 1/ The amount of food fed was based on the trout feeding tables of Deuel, Haskell, Brockway, and Kingsbury (1952). As these tables base the amount of food fed on fish size at a given water temperature, the amount fed in percentage of the body weight differed for the various size groups of fish.

These experiments were run for 16 weeks: 8 weeks after the initial grading and 8 weeks after the final grading. Data are summarized in tables 1, 2, and 3. In table 1, the weights of the graded groups of fish have been recombined at the end of 8 weeks and 16 weeks; in table 2, for the total 16 weeks. The total amount of food fed to the fish is listed in these tables for both the graded and the ungraded groups.

1/ Diet composition: 25 percent each of beef liver and beef spleen; 12 percent each of white fish meal, cottonseed meal, distiller's solubles, and wheat middlings; 2 percent salt. After being mixed, the diet was passed through a centrifugal blower.

The average weights of the individual trout listed in table 3 provide a comparison of the growth rates of the fish in the size groups. Significance of all comparisons was established by analysis of variance.

Discussion

At the end of the first 8 weeks of the experiments, the recombined weights, the total percent gains, and the food conversions into fish flesh of the graded groups did not differ from similar statistics of the ungraded fish.

In percent of the body weight, the amounts of food fed were: small graded fish, 8.0 percent; large graded fish, 6.5 percent; and ungraded fish, 7.0 percent. The total amounts of food fed the graded and ungraded groups were almost identical, and the food conversions were equal (2.6 pounds of food required per pound of fish produced). Since the selection of food levels varied with fish size (water temperature was equal in all groups), and since the total amount of food fed to the small and large graded trout equalled the total amount fed the ungraded trout, these results may be considered as confirmation of the validity of the feeding tables of Deuel et al. (1952).

The results of the second 8 weeks of the experiment, during which time the graded fish were divided into three sizes, were similar to those of the first 8 weeks. There was no significant difference between the total weights of the graded and ungraded fish groups. The percent gain of the graded fish (111 percent) was equal to that of the ungraded (112 percent). The food fed and the food conversions were equal for the two groups. The food conversions were less efficient for the second 8-week period than for the first, an indication of over-feeding during the latter part of the experiment.

Over the 16-week period, the recombined weights of the graded trout and the total weight of the ungraded were similar and there was no significant difference in the percent gains. The food conversions were identical (3.5), and there was no significant difference in the total food fed.

TABLE 1.--Weights, food fed, and food conversion of graded and ungraded populations of brook trout during two 8-week experimental periods

Experiment number[1/]	Total weight at start (grams)	Weights at end of period: Small trout (grams)	Medium trout (grams)	Large trout (grams)	Total weight at end (grams)	Total percent gain	Total food fed (in grams) to: Small trout	Medium trout	Large trout	Total food fed (grams)	Total conversion
				At end of first 8 weeks							
1-graded--	2,009	2,400	-----	4,583	6,983	238	5,414	-----	8,335	13,749	2.76
2-graded--	2,042	2,194	-----	5,263	7,457	266	5,247	-----	9,851	15,097	2.80
3-graded--	1,978	1,804	-----	5,524	7,328	270	4,260	-----	9,797	14,057	2.62
4-graded--	1,937	2,810	-----	4,800	7,610	292	5,735	-----	8,219	13,954	2.46
Average	1,992	-----	-----	-----	7,344	269	-----	-----	-----	14,213	2.65
1-ungraded	2,000	-----	-----	-----	7,705	286	-----	-----	-----	14,621	2.56
2-ungraded	2,000	-----	-----	-----	7,053	274	-----	-----	-----	13,763	2.71
3-ungraded	2,000	-----	-----	-----	7,400	270	-----	-----	-----	14,451	2.67
4-ungraded	2,000	-----	-----	-----	7,030	252	-----	-----	-----	13,936	2.77
Average	2,000	-----	-----	-----	7,297	264	-----	-----	-----	14,193	2.64
				At end of second 8 weeks							
1-graded--	6,784	4,203	3,016	6,855	14,074	110	8,847	6,941	13,640	29,428	4.00
2-graded--	7,168	3,487	4,067	7,296	14,850	107	7,399	9,265	14,530	31,194	4.05
4-graded--	7,553	5,612	2,394	7,472	15,478	116	12,436	5,740	12,245	30,421	3.68
Average	7,168	-----	-----	-----	14,800	111	-----	-----	------	30,348	3.90
1-ungraded	7,704	-----	-----	-----	16,111	109	-----	-----	------	34,967	4.15
2-ungraded	7,053	-----	-----	-----	15,039	113	-----	-----	------	32,237	4.04
3-ungraded	7,400	-----	-----	-----	15,350	108	-----	-----	------	32,982	4.14
4-ungraded	7,030	-----	-----	-----	15,177	116	-----	-----	------	32,242	3.96
Average	7,297	-----	-----	-----	15,419	112	-----	-----	------	33,142	4.06

[1/] No. 3-graded was lost during a storm in the second 8 weeks of the experiment.

TABLE 2.--Weights, food fed, and food conversion of graded and ungraded populations of brook trout during a 16-week experimental period

Experiment number[1/]	Total weight at start (grams)	Weights at end of period: Small trout (grams)	Medium trout (grams)	Large trout (grams)	Total weight at end (grams)	Total percent gain	Total food fed (in grams) to: Small trout	Medium trout	Large trout	Total food fed (grams)	Total conversion
1-graded--	2,009	4,203	3,016	6,855	14,074	600	14,261	6,941	21,975	43,227	3.6
2-graded--	2,042	3,487	4,067	7,296	14,850	627	12,646	9,265	24,381	46,292	3.5
4-graded--	1,937	5,612	2,394	7,422	15,478	691	18,171	5,740	22,464	46,373	3.4
Average	1,996	-----	-----	-----	14,801	639	------	-----	------	45,298	3.5
1-ungraded	2,000	-----	-----	-----	16,111	705	------	-----	------	49,588	3.5
2-ungraded	2,000	-----	-----	-----	15,039	651	------	-----	------	46,000	3.5
3-ungraded	2,000	-----	-----	-----	15,350	667	------	-----	------	47,433	3.5
4-ungraded	2,000	-----	-----	-----	15,177	658	------	-----	------	46,178	3.5
Average	2,000	-----	-----	-----	15,419	670	------	-----	------	47,299	3.5

[1/] No. 3-graded was lost during a storm in the second 8 weeks of the experiment.

TABLE 3.--Average weights and percent gains of graded and ungraded populations of brook trout during two 8-week experimental periods

Experiment number[1/]	Graded small trout			Graded medium trout			Graded large trout			Ungraded trout		
	Average weight at start (grams)	Average weight at end (grams)	Percent gain	Average weight at start (grams)	Average weight at end (grams)	Percent gain	Average weight at start (grams)	Average weight at end (grams)	Percent gain	Average weight at start (grams)	Average weight at end (grams)	Percent gain
					At end of first 8 weeks							
1	3.34	11.29	238	----	----	---	5.46	20.41	274	4.82	19.2	298
2	3.68	13.35	264	----	----	---	6.25	25.60	310	4.82	17.3	259
3	3.53	12.70	260	----	----	---	5.45	20.50	276	4.82	17.8	269
4	3.97	15.45	289	----	----	---	6.15	23.50	283	4.82	18.7	287
Average	3.63	13.20	262	----	----	---	5.83	22.50	286	4.82	18.3	278
					At end of second 8 weeks							
1	9.95	23.7	138	14.5	28.7	98	24.6	49.7	102	19.2	40.6	112
2	11.6	28.6	147	16.9	33.0	96	25.3	50.3	99	17.3	42.3	144
3	-----	----	---	----	----	--	----	----	---	17.8	37.6	111
4	12.85	30.6	138	20.0	39.2	96	28.3	61.5	117	18.7	40.8	118
Average	11.47	27.6	141	17.1	33.6	97	26.1	53.8	106	18.3	40.3	121

[1/] No. 3-graded was lost during a storm in the second 8 weeks of the experiment.

In these experiments, grading a fish population did not increase the weight of fish produced or the efficiency of the food conversion. On the basis of the average weight of individual fish (table 3), there was no significant difference in the rates of gain for the small graded, large graded, or ungraded trout during the first 8 weeks. During the second 8 weeks, the small graded trout grew at the fastest rate. The ungraded trout gained at a slower rate than the small graded fish, but faster than either the medium or the large graded fish. There was no difference between the medium and the large graded fish.

During the second 8 weeks there was sufficient difference in the size groups of graded fish to produce a measureable difference in the percentage gain of the small trout. The recombined weights of the graded fish, however, did not exceed the weights of the ungraded trout; and therefore the grading did not increase the over-all production of these lots of fish.

The mortalities for the various groups of fish have been summarized in table 4. Variation within treatments reduced the precision of measurement of differences, but there was a slight decrease in total mortality of the graded trout. There was no significant difference in the mortality rates of the size groups of the graded trout during either of the 8-week periods.

The mortality rates in table 4 are based on observed losses and therefore do not include losses that might have been caused by cannibalism. Previous studies at this laboratory have shown that cannibalism is not a problem in our studies on brook trout, though it seriously affects studies on brown trout (Phillips et al. 1954). Observed losses indicate that there was a slightly greater survival of the graded trout, but the small difference would not materially affect the production of fish flesh over the experimental period.

These results do not support Brown's conclusions (1946), but agree in general with those of Haskell (1959) and R. E. Burrows[2], both of whom failed to show

TABLE 4.--Observed percent mortalities of graded and ungraded populations of brook trout during two 8-week periods and during the total 16 weeks

Experiment number[1]	Percent mortality			
	Small trout	Medium trout	Large trout	Total population
At end of first 8 weeks				
1-graded--	3.2	---	3.9	3.5
2-graded--	1.8	---	3.0	2.5
3-graded--	4.1	---	3.1	3.4
4-graded--	1.6	---	2.6	2.1
Average	2.7	---	3.2	2.9
1-ungraded	---	---	---	4.5
2-ungraded	---	---	---	1.9
3-ungraded	---	---	---	4.8
4-ungraded	---	---	---	4.8
Average	---	---	---	4.0
At end of second 8 weeks				
1-graded--	0.0	2.1	0.0	0.5
2-graded--	.8	1.6	.0	.8
4-graded--	1.1	3.9	4.0	2.7
Average	1.0	2.5	1.3	1.3
1-ungraded	---	---	---	0.7
2-ungraded	---	---	---	2.5
3-ungraded	---	---	---	1.5
4-ungraded	---	---	---	2.3
Average	---	---	---	1.7
Total 16-week period				
1-graded--	---	---	---	4.3
2-graded--	---	---	---	3.3
4-graded--	---	---	---	5.0
Average	---	---	---	4.2
1-ungraded	---	---	---	5.3
2-ungraded	---	---	---	4.3
3-ungraded	---	---	---	6.2
4-ungraded	---	---	---	6.9
Average	---	---	---	5.7

[1] No. 3-graded was lost during a storm in the second 8 weeks of the experiment.

[2] Bureau of Sport Fisheries and Wildlife, Entiat, Wash. Personal communication, 1961.

value in grading fish in terms of growth rate of the fish. It is possible that the present work (on brook trout) and that of Haskell (on brook and brown trout) and Burrows (on salmon) did not concern submissive groups of small fish from a well-established population hierarchy. Fish size alone is probably not a valid criterion of a submissive individual, as smallness may also be an inherited or otherwise imposed trait. If the latter is the situation in the present studies (using fish populations), these results are not necessarily at odds with those of Brown (using individuals). It is also possible that experimental methods, types of food, or methods of feeding may explain in part some of the observed differences of the present work and that of Brown.

The value of grading trout to meet fishery-management requirements or to improve certain hatchery operations is not questioned. However, for brook trout of the sizes used in these studies, grading to increase the weight of fish produced or the efficiency of food conversion is a questionable hatchery practice.

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ALASKA BLACKFISH RECEIVED BY NATIONAL AQUARIUM

Fifty Alaska blackfish (Dallia pectoralis) were trapped in the tundra bogs near Nome, Alaska, by personnel of the Bureau of Sport Fisheries and Wildlife Service. Placed in a 300-gallon distribution tank that was supplied by the Alaska Department of Fish and Game, the fish were carried to a waiting Air Force plane. Within a few hours they were swimming in tanks at the National Aquarium, Washington, D. C.

Previous shipments of Alaska blackfish to the National Aquarium have proved popular with the visiting public, arousing much interest. The fish live well in captivity. At the Aquarium they are being fed a diet of sheep liver sliced into worm-like pieces.

For a long time the Alaska blackfish has been the subject of much controversy, especially in regard to its reported ability to survive being frozen in ice. The fish is frequently found in shallow ponds, and it is known to survive for extended periods in moist tundra mosses.

Recent surveys have shown that the brown trout, unlike the rainbow and brook trout, is almost completely free from neoplastic and degenerative diseases.

THE INFLUENCE OF VARIOUS DIETS ON TROUT GROWTH AND PIGMENTATION

L. P. Bouthillier
Department of Biochemistry, University of Montreal
Montreal, Quebec, Canada

THIS WORK was undertaken at the Laurentian Hatchery, St. Faustin, Province of Quebec, for the purpose of finding a diet which would promote satisfactory growth and pigmentation of the brook trout (Salvelinus fontinalis).

We have studied the relative nutritive properties of 18 different diets that are fully described in table 1. It should be observed that beef liver was used as the standard comparison diet.

Carefully selected young brook trout fingerlings were used in all our feeding experiments. The fingerlings were divided into 18 lots, each containing about 555 fish. Individual troughs were used. The dimensions of the troughs were as follows: total length, 10 feet; inside width, 18 inches; depth, 8 inches, with 6 to 6.25 inches of water.

Cold spring water of excellent quality was run through the troughs. As it is well known that fish kept in cold water show little appetite, it became important to warm the water slightly before it reached the troughs. The water was heated during the day with the aid of a special furnace.

We wished to establish the relative nutritive value of the assay diets, and took care to study their growth-promoting properties by considering equal weights of the dried diets. In the last column of table 1 are given the percentages of dry matter contained in the various diets.

In most hatcheries, it is accepted that an amount of beef liver equivalent to 10 percent (calculated on wet basis) of the weight of trout proves to be entirely satisfactory for promoting the optimal growth of the fish. This value was taken into account in calculating the daily allowances of the various diets. We found that 10 grams of beef liver supplied 2.76 grams of dry matter. Consequently, each other diet was fed daily to a particular lot of fish in such a quantity (wet basis) that it brought 2.76 grams of dry matter per 100 grams of trout. One portion of the day's food was served in the morning, and the other late in the afternoon; each portion of food was spread over a metal tray which was put under water.

Once a week, each lot of trout was weighed according to the following method: The fish were put in a wire basket and allowed to drain for 3 to 5 seconds. Then the fish were emptied into a pail of water on a set of torsion-balance scales and weighed. The manipulative error of this technique has been less than 1 percent.

In the first series of feeding experiments, the growth-promoting qualities of diets A, B, C, D, E, F, G, H, J, and K were established. Figure 1 shows the growth made on each of these diets, as well as the weekly gain in weight per 100 grams of trout.

The data concerning diets A and B show that the use of iodine as a supplement promotes trout growth. When the results obtained from diets A and B are compared with the results from the other diets (which contained the same supplement of iodine), there seems to be justification for concluding that carrots, casein, fish meal, fresh fish, fox food, whole wheat

TABLE 1.--Composition of diets

Diet	Ingredients	(grams)	(milliliters)	Dry extract (percent)
A	Beef liver	100.0	----	27.4
B	Beef liver	100.0	----	
	Iodine solution[1]	-----	0.1	27.4
C	Beef liver	82.0	----	
	Fresh carrots	14.5	----	
	Water	-----	3.5	
	Iodine solution[1]	-----	.1	23.9
C-m	Beef liver	82.0	----	
	Fresh carrots	14.5	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	24.7
D	Beef liver	70.0	----	
	Commercial casein	15.0	----	
	Water	-----	15.0	
	Iodine solution[1]	-----	.1	33.3
D-m	Beef liver	70.0	----	
	Commercial casein	15.0	----	
	Water	-----	11.5	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	34.1
E	Beef liver	71.5	----	
	Fish meal	25.0	----	
	Water	-----	3.5	
	Iodine solution[1]	-----	.1	40.7
E-m	Beef liver	71.5	----	
	Fish meal	25.0	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	41.5
F	Beef liver	48.3	----	
	Fresh fish	48.3	----	
	Water	-----	3.4	
	Iodine solution[1]	-----	.1	26.2
F-m	Beef liver	48.3	----	
	Fresh fish	48.3	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	27.0

TABLE 1.--Composition of diets--Continued

Diet	Ingredients	(grams)	(milliliters)	Dry extract (percent)
G	Beef liver	48.3	----	
	Fox food	48.3	----	
	Water	-----	3.4	
	Iodine solution[1]	-----	.1	24.9
G-m	Beef liver	48.3	----	
	Fox food	48.3	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	25.7
H	Beef liver	82.0	----	
	Whole wheat flour	14.5	----	
	Water	-----	3.5	
	Iodine solution[1]	-----	.1	35.7
H-m	Beef liver	82.0	----	
	Whole wheat flour	14.5	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	36.5
J	Beef liver	85.0	----	
	Fresh clover leaves	11.5	----	
	Water	-----	3.5	
	Iodine solution[1]	-----	.1	24.9
J-m	Beef liver	85.0	----	
	Fresh clover leaves	11.5	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	25.7
K	Beef liver	71.5	----	
	Shrimp meal	25.0	----	
	Water	-----	3.5	
	Iodine solution[1]	-----	.1	40.6
K-m	Beef liver	71.5	----	
	Shrimp meal	25.0	----	
	Salt solution I[2]	-----	1.0	
	Salt solution II[2]	-----	2.0	
	Calcium carbonate	.4	----	
	Iodine solution[1]	-----	.1	41.3

[1] Containing 5 percent potassium iodide and 1 percent free iodine.

[2] For composition, refer to table 2.

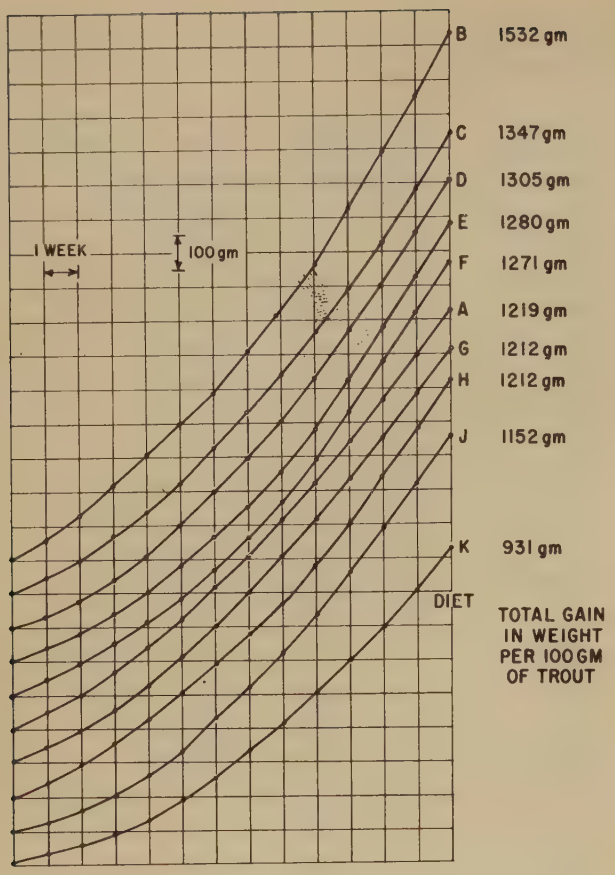

FIGURE 1.--Comparison of growth of trout fed on diets A, B, C, D, E, F, G, H, J, and K, and the weekly gain in weight per 100 grams of trout.

flour, clover leaves, and shrimp meal all possess a lower nutritive value than beef liver. Nevertheless, the results demonstrate clearly that trout may grow fairly well when fed on most of the diets studied.

Trout that were fed diet K, which contained a fairly good amount of shrimp meal, showed remarkable pigmentation of the fins. On the other hand, the fish that received diet F, which contained beef liver and fresh fish in equal amounts, developed beautiful pigmentation on the body.

In the same series of experiments, we studied the nutritive value of each assay diet supplemented with 1 percent of the Jones-Foster salt mixture. The latter preparation is described in table 2.

TABLE 2.--Composition of the salt mixture[1/]

Salt solution		Grams
I	$NaCl$	290.0
	$MgSO_4$	120.0
	$FeSO_4$, $7H_2O$	56.0
	$MnSO_4$, $4H_2O$	11.2
	$CuSO_4$, $5H_2O$	1.0
	$ZnCl_2$	.5
	$CoCl_2$, $6H_2O$	.05
	H_2O ad	2,097 milliliters
II	KH_2PO_4	815.0
	KI	2.0
	NaF	1.5
	H_2O ad	4,194 milliliters

[1/] 1 milliliter salt solution I + 2 milliliters salt solution II + 0.4 gram calcium carbonate = 1 gram of salt mixture, as described by J. H. Jones and Claire Foster (1942), Journal of Nutrition, 24 (3), 245-256.

Figure 2 shows the growth made by trout fed on diets C-m, C, D-m, D, E-m, E, F-m, and F, as well as the weekly gain in weight per 100 grams of trout. Figure 3 presents this information for diets H-m, H, G-m, G, J-m, J, K-m, and K. It is to be observed that in seven cases out of eight, supplementation with mineral salts proved to be only slightly beneficial. Because of small differences in growth registered, there seems to be no justification for concluding that the addition of a certain amount of mineral salts into the diets improves their nutritive quality.

On the basis of the findings in the first series of experiments, we prepared what may be called a special diet, in which were grouped the food substances that had a good nutritive value and promoted pigmentation. We wish to point out that the cost of this diet is less than half that of beef liver. The composition of the special diet is presented in table 3. This diet contained 37.5 percent of dry matter.

In a second series of experiments, we compared the growth-promoting properties

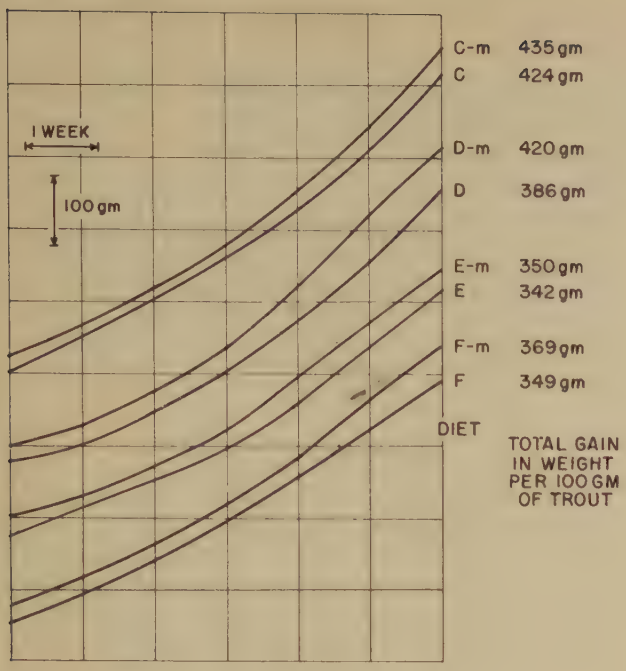

FIGURE 2.--Comparison of growth of trout fed on diets C-m, C, D-m, D, E-m, E, F-m, and F, and weekly gain in weight per 100 grams of trout.

of the special diet and the beef liver (supplemented with iodine), feeding each at different levels of intake calculated on dry basis. Each of four lots of fingerlings received the beef liver diet in daily portions representing, respectively, 14, 12, 10, and 8 percent (on wet basis) of the weight of the fish, or an equivalent of 3.86, 3.31, 2.76, and 2.21 grams of dried beef liver per 100 grams of trout. At the same time, five other lots of fish received daily feedings of 4.42, 3.86, 3.31, 2.76, and 2.21 grams, respectively, of the special diet (dry basis) per 100 grams of fingerlings.

Figure 4 shows the growth that was made by trout receiving the special diet and the beef liver diet. These data indicate that the beef liver diet possesses a higher nutritive value than the special diet. However, the optimal growth of the trout was reached when the beef liver was fed daily to the level of 10 percent of the weight of trout, or an equivalent of 2.76 grams of dried beef liver per

TABLE 3.--Composition of the Special Diet

Ingredients	Grams	Milliliters
Beef liver---------	30	---
Fresh fish---------	30	---
Fox food----------	20	---
Whole wheat flour--	15	---
Shrimp meal--------	5	---
Iodine solution----	--	0.1

100 grams of fish. On the other hand, these fish grew just as well when they were given an equivalent of 3.86 grams of the dried special diet per 100 grams of trout.

As we expected, the trout that were fed the special diet showed a beautiful pigmentation which could not be found on the bodies of trout that were given the beef liver diet.

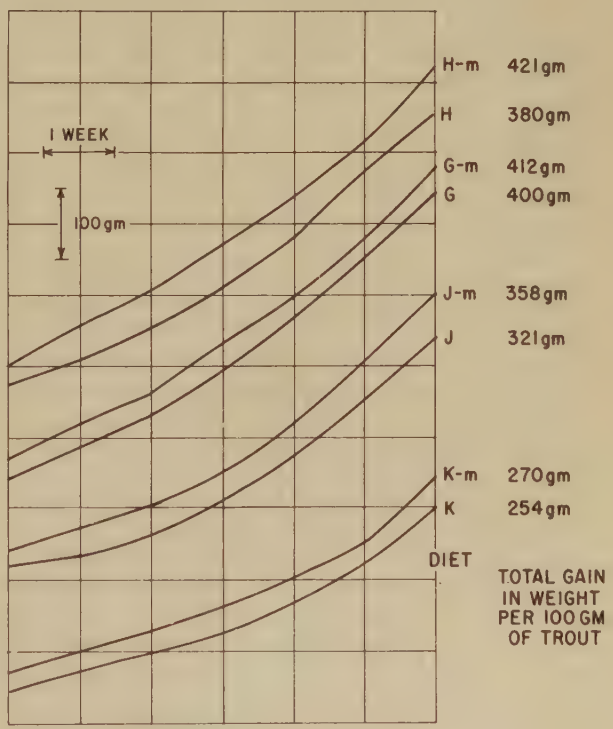

FIGURE 3.--Comparison of growth of trout fed on diets H-m, H, G-m, G, J-m, J, K-m, and K, and the weekly gain in weight per 100 grams of trout.

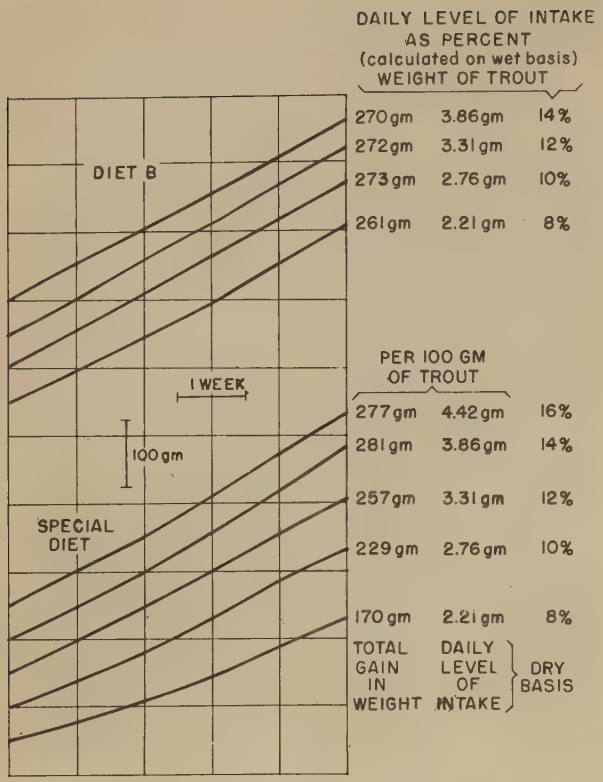

FIGURE 4.--Comparison of growth of trout fed different levels of intake of diet B and the Special Diet, and the weekly gain in weight per 100 grams of trout.

Summary

Using the growth method, trout feeding experiments have been conducted to obtain definite information concerning the nutritive value of certain diets. The addition of a salt mixture to the assay diets improved their nutritive quality very little if at all. An inexpensive diet which promoted good growth and excellent pigmentation of trout is proposed.

Acknowledgments

I am deeply indebted to Dr. Lucien Piché, former director of the Laurentian Hatchery, for his helpful advice throughout these studies. I wish to thank also Dr. Gustave Prévost, director of the Office de Biologie de la Province de Quebec, and Mr. B. W. Taylor, director of fish culture, Department of Fish and Game, Province of Quebec, who furnished material facilities for the conduct of this research.

TRICAINE METHANESULFONATE AS AN ANESTHETIC FOR HERRING

Dissolved at 1:20,000 in sea water of 30 parts per thousand and 8° C. temperature, tricaine methanesulfonate effectively anesthetized 5-inch (total length) herring, Clupea harengus, in about 8 minutes.

In the first few minutes of immersion in the solution the test fish gradually became motionless, but when touched responded instantly by swimming: even a light tap on the aquarium wall caused a brief effort to swim. When there was no response to a gentle tap, anesthesia was judged to be total. The fish then offered no resistance to handling. The time required by the fish to resume a normal swimming attitude after being returned to untreated circulated sea water was about equal to the time required to induce anesthesia. If a fish was left in the solution for 3 or 4 minutes after having reached total anesthesia, death was almost certain. Escapement of gas bubbles from the vent was the first sign that the fish could not be revived.

Four groups of 10 fish each were anesthetized and are considered to have wholly recovered, recovery being defined as survival for 1 week after anesthetization. Test and control fish were taken from the same immature school; a fresh solution of anesthetic was mixed for each lot tested. Test fish were identified by fin-clipping.

--JOHN E. WATSON, Bureau of Commercial Fisheries, Biological Laboratory, Boothbay Harbor, Maine.

HATCHERY PRODUCTION EXPERIMENT DESIGNED TO PREVENT SUNBURN IN LANDLOCKED SALMON

Bernard W. Corson and Peter E. Brezosky
New Hampshire Fish and Game Department
Concord, New Hampshire

A SERIES OF EXPERIMENTS was designed and completed to establish the production values of basic preliminary tests made by DeLong, Halver, and Yasutake (1958) on the control of sunburn symptoms.

During the past 10 years, the New Hampshire Fish and Game Department, working on the premise that larger fish have a higher rate of survival when they are planted in open waters, has produced larger landlocked salmon (Salmo salar) for stocking purposes. Although improved diets and a reduction in carrying capacities have produced larger fish, climatic conditions have made it necessary to hold the salmon until they are 14 to 18 months old before stocking them.

During the 14- to 18-month retention period, a mortality of 25 to 40 percent could be anticipated in the Powder Mill Rearing Station's yearling salmon as the result of sunburn, which caused open lesions on the skull and secondary infections extending to the posterior dorsal areas (figure 1).

This problem with sunburn symptoms occurs in only one of the three salmon production units in New Hampshire. The

FIGURE 1.--A salmon showing the effects of sunburn.

New Hampton and Warren hatcheries rear salmon through the 14- to 18-month period without any of the symptoms of sunburn. This may be attributed to the amounts of tannic acid compounds present in these hatching waters. The presence of these compounds and their relation to the melanin concentration of the salmon dermis and its protection against ultra-violet rays of the sun have been reviewed. If tannin compounds do react accordingly, there is no practical application to duplicate this condition at the Powder Mill Rearing Station, where tests indicate that these compounds are very limited and the symptoms of sunburn are prevalent.

It was impossible, under the current hatchery management program, to hold additional numbers of salmon at the New Hampton and Warren hatcheries. Powder Mill, the largest of New Hampshire's eight stations, was confronted with the problem of sunburn symptoms during the second

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DeLong, Halver, and Yasutake (PROGRESSIVE FISH-CULTURIST 20 (3): 111-113) reported that sunburn in fish could not be prevented by feeding niacin, and that use of this vitamin as a supplement to the diet could not be expected to lower the incidence of sunburn unless the diet had previously been deficient in niacin and/or tryptophan.--The EDITORS.

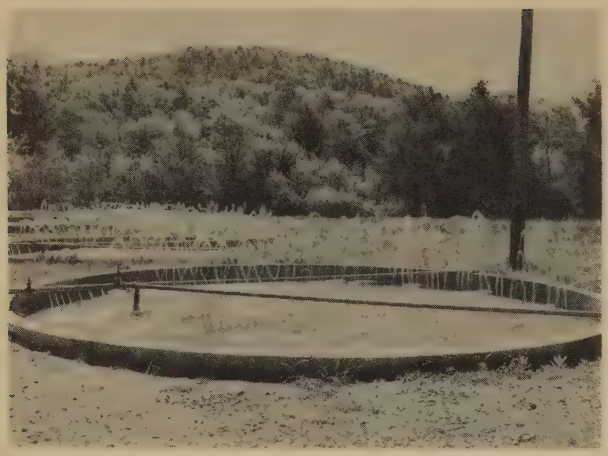

FIGURE 2.--A pool equipped with the spray system.

summer of the salmon. It was, then, unanimously agreed that the only real test would be to conduct the cultural research on a production basis at this unit.

The project covered a 2-year period: part I was completed in 1959; part II, in 1960.

Part I

Early in the spring of 1959 the experiment was designed to test three procedures: (a) Using a spray system to break the upper layer of water and thereby deflect the sunrays, (b) covering pools with saran cloth for a 90-percent calculated shade to prevent direct sunlight, and (c) supplementing the salmon diet with niacin in reasonable amounts without causing physiological damage.

All the pools used in these tests were identical.

Water Spray.--On May 1, 1959, each of three concrete circular pools, 25 feet in diameter, was equipped with two 2-inch pipes that reached completely across it. With 3/16-inch holes bored into them at 4-inch intervals, these pipes provided the pools with adequate spray (figure 2).

Three lots of 5,000 yearling salmon weighing 62 per pound were put into these pools on May 1, 1959. Before the introduction of the salmon, a few had exhibited preliminary symptoms of sunburn.

After a period of 1 month the salmon in all three pools were seriously affected by sunburn and a secondary fungus infection.

Mortalities during May amounted to 12.1 percent. During the first 2 weeks of June, an additional 3.1 percent mortality occurred; thereafter, the effects of sunburn rapidly disappeared. It was difficult to believe that the badly affected fish would recover and heal, but such was the case.

During the last week in June and during July the only evidence of sunburn was a completely healed dark area on the heads where the skull had actually been exposed a few weeks before.

Shade.--On May 1, 1959, three circular pools were covered with saran cloth providing 90 percent shade (figure 3). Five thousand yearling salmon weighing 62 per pound were placed in each shaded pool.

The May and June mortalities totaled 3.4 percent. This loss was not characterized by the symptoms of sunburn.

Salmon Diet Fortified With Niacin.--Three lots of 5,000 yearling salmon that weighed 62 per pound were introduced into three circular pools on May 1, 1959.

The salmon rations were supplemented with pure niacin in amounts equal to 0.5 pound per ton of mixed diet. Standard

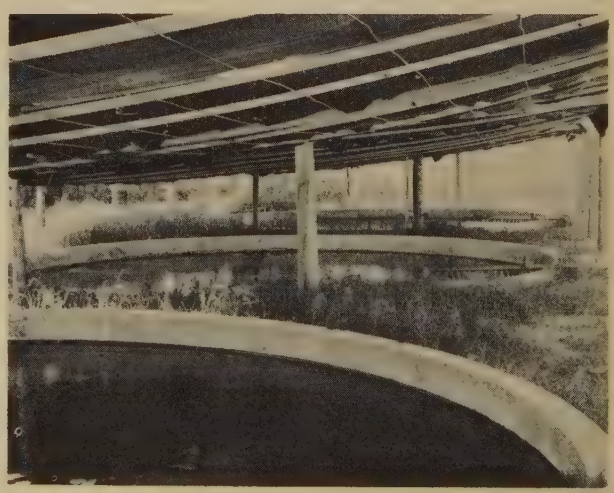

FIGURE 3.--Pools shaded with saran cloth.

feeding frequencies, as well as normal daily requirements, were maintained.

The mortalities during May and June totaled 16.7 percent. It was again observed in this series of tests that symptoms of sunburn rapidly disappeared in July. The remaining experimental salmon were retained on this niacin-fortified diet until they were 18 months old without any apparent physiological damages.

Control Unit.--On May 1, 1959, one lot of 5,000 yearling salmon weighing 62 per pound was placed in a circular pool to serve as a control.

Mortalities totaled 11.6 percent for May and the first 2 weeks of June. The symptoms of sunburn were greatly retarded thereafter.

Part II

The procedure at the Powder Mill Rearing Station is to hold the salmon in raceways until they are spring yearlings. When the water temperature rises, they are transferred into circular pools (described in part I) with a separate intake and discharge.

The second series of production tests was designed to start before the development of any indication of sunburn on the experimental and control salmon. The salmon used in these tests were approximately 12 months old.

Use of the spray system to control symptoms of sunburn had proved completely inefficient and was discontinued in the 1960 production experiments.

Shade.--Two lots of 6,000 salmon that weighed 75 per pound were placed in two concrete raceways measuring 50 by 8 feet. Eighty percent of the surface area of these raceways was covered with saran cloth on February 17, 1960. For a control, 6,000 salmon were placed in an adjacent raceway without shade.

No evidence of a sunburn condition existed in the two shaded raceways at the end of May. The mortality from February 17 to May 31 totaled 1.7 percent.

Salmon in the third raceway became severely sunburned, and open lesions on the dorsal surface of the head extended posteriorly along the back. Mortalities in the control unit for the same period totaled 11.5 percent.

Salmon Diet Fortified With Niacin.--On March 14, 1960, three additional 50-by 8-foot concrete raceways--referred to as A, B, and C--were set up to carry 3,500 salmon (approximately 13 months old) in each. The diet in raceway A was supplemented on the basis of 1.1 pounds of pure niacin per ton of salmon rations. The fish in raceway B received pure niacin at the rate of 2.2 pounds per ton of rations. The fish in raceway C served as a control unit, receiving the standard salmon rations without being fortified with niacin.

Although the diets of salmon in raceways A and B were fortified with niacin before there was any evidence of sunburn, definite sunburn symptoms were observed in both lots of fish. Raceways A and B had mortalities of 10.8 and 11.0 percent, respectively. Raceway C, the control, suffered an 18.9-percent mortality.

The use of niacin did not prevent the appearance of these symptoms of sunburn and demonstrated that there was little advantage in adding nicotinic acid to the rations.

Summary

Three different experiments on yearling landlocked salmon were conducted in complete production units at the Powder Mill Rearing Station in an effort to find a means of preventing sunburn.

The spray system was completely ineffective and was discontinued after October 1959.

Pure niacin used as a supplement in the amounts of 0.5, 1.1, and 2.2 pounds per ton of salmon rations had no positive results and did not prevent the appearance of sunburn.

The provision of shade over salmon pools from February 17 to May 31 had very positive results. None of the salmon in these pools developed symptoms of sunburn.

Sunburn on yearling salmon appears early in April and is followed by heavy

mortalities during May and through the first half of June. After this period the condition regresses and the salmon fully recover.

The results of this study indicate that an expected mortality of 25 to 40 percent at the Powder Mill Rearing Station can be eliminated by using shade as a fish-cultural tool. This will enable the Fisheries Division to hold a predetermined quota of yearling salmon until conditions in the lakes and their tributaries are favorable for stocking.

Acknowledgments

The authors wish to express sincere thanks to Superintendents Arthur Butler and Frank Seaver and the fish-culturists at the Powder Mill Rearing Station, New Durham, New Hampshire.

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TOXICITY OF AN EPOXY CEMENT TO FISHES

In tests of components of the water system at this laboratory, epoxy material used to cement fiberglass screen holders to experimental troughs proved toxic to certain fishes. The cement consisted of an epoxy resin, an amide hardener, and methyl ethyl ketone as a thinner.

Tests were made to find a safe procedure for its use. Five-gallon jars were filled with 15 liters of reconstituted demineralized water (pH, 7.1; alkalinity, 35) and put in a circulating water bath at 13° C. The cement was painted on two fiberglass rods (0.5 by 0.5 by 13 inches) which were then suspended in the test jars. Two controls were used in each test: one jar had two untreated rods; the other, none. Each test was repeated at least once.

In the first test the cement dried on the rods 4 days to a firm but slightly "tacky" state. Of three test jars, one received 12 rainbow trout fry (average standard length, 25 millimeters); one, 6 rainbow fingerlings (51 millimeters); and one, 12 goldfish (39 millimeters). The rainbow fry were dead in 3 hours, the rainbow fingerlings in 8, and the goldfish in 18 hours. No deaths were recorded in the controls.

Two more tests used cement that had dried 4 days and fish from the same three lots mentioned. In one test, treated rods were flushed in running well water for 2 weeks before being put in the jars. This apparently removed the toxicant, as no fish died. In the other test, the fish were put in a fiberglass trough with 15 liters of well water changing at the rate of 4 liters a minute. The treated rods were put on the bottom of the trough, The change of water apparently prevented a lethal build-up of a toxic substance, as no fish died.

Last, a test was run on cement that had dried for 1 month on the rods to complete hardness. The 12 rainbow fingerlings (28 millimeters) used to test this cement survived a 50-hour exposure but died rapidly thereafter. No control fish was affected.

Epoxy cements probably differ in toxicity, but all are dangerous in a closed system unless it is well flushed before use. Even in a constantly changing water system, cement should be thoroughly hardened and well flushed before it is used.

--DONALD F. MAIRS, Bureau of Sport Fisheries and Wildlife, Fish Control Laboratory, La Crosse, Wisconsin.

THE EFFECT OF THYROID-STIMULATING HORMONE COMBINED WITH GONADOTROPHIC HORMONES ON THE OVULATION OF GOLDFISH AND GREEN SUNFISH

Kermit E. Sneed and Harry K. Dupree
Bureau of Sport Fisheries and Wildlife
Southeastern Fish-Cultural Laboratory, Marion, Alabama

THE TECHNIQUES EMPLOYED TO INDUCE OVULATION IN FISHES usually involve the injection of fish pituitaries (Pickford and Atz 1957) or human chorionic gonadotrophin (Sneed and Clemens 1959, Ramaswami and Sundararaj 1956, Ramaswami and Lakshman 1958). The collection and preparation of fish pituitaries is laborious, and commercial chorionic gonadotrophin is expensive. Also, these materials sometimes require the use of multiple-injection procedures that consume valuable time and labor. Even when the fish-culturist selects fish that appear gravid and ready to spawn, the results are often variable; that is, the fish which appear to be in the same physiological condition differ in receptivity to hormone injections.

It is desirable from a fish-culturist's viewpoint to achieve greater spawning success. Also, it would be interesting from a biologist's viewpoint to explain the differences in response of individuals of the same group of fish which appear alike in sexual development. Some questions and possible answers, therefore, are in order.

1. Do some fish fail to respond because of incomplete sexual development? This is the first and most logical question. Sneed and Clemens, in most of their papers on induced spawning, have emphasized the importance of selecting ripe fish. When such selections are made, the percentage of successful spawns is almost always higher than if random injections are made. Obviously, such selections are not perfect, as less than 100 percent of the chosen fish spawn after injections.

2. Are some of the fish of a population physiologically deficient in some respect not obvious to the fish-culturist? Do some fish have, for example, hypothyroidism or low metabolic rate, avitaminosis, low pituitary activity or other endocrine dysfunction?

3. Do fish (of the same species) vary greatly in their requirement for the hormones, and therefore is a different dosage or different ratio of components required for each fish?

Obviously, other questions can be asked, but those listed indicate the problem. After several years of experience and many experiments, we think that the percentage of fish responding successfully to the injection of gonadotrophins depends on the species of fish, season, gonadal development, water temperature, physiological condition of the fish, and perhaps on the composition and quantity of components in the gonadotrophic materials. Some of these conditions can be influenced in varying degrees by the investigator, but most of these factors cannot be controlled or even evaluated. However, there is a possibility that a gonadotrophic material or a combination of gonadotrophic and nongonadotrophic materials can be devised

to induce complete ovulation at dosages near the physiological levels required for normal ovulations, thus, in a sense, minimizing some of the variables in the environment and in the physiology of the fish.

Ramaswami and Sundararaj (1956) induced spawning in the Indian catfish with large dosages of thyroid-stimulating hormone (TSH) and also with this material in combination with gonadotrophins. Induced ovulation with high dosages of TSH may have been due to contamination of the material with gonadotrophins, but possibly the effect on reproduction is real--the initial effect being an increase in basal metabolism or increased activity of the fish's own pituitary. In support, Albert (1945; reviewed by Pickford and Atz 1957) found that single intraperitoneal injections of purified sheep TSH affected the thyroid gland of Fundulus as follows: no change in 3 hours; slight change after 6 hours; increased cell height, increased vascularization, and less colloid after 24 hours; and marked hypertrophy and hyperplasia after 3 days. Berg and Gorbman (1954; reviewed, Pickford and Atz 1957) reported that when 5 units of purified TSH was injected daily for 4 or 5 days in goldfish, Carassius auratus, there was an increase in iodine uptake and increased synthesis of thyroxin as compared with the controls. Also, Kyle et al. (1954; reviewed, Pickford and Atz 1957) concluded that TSH had no effect on thyroid-ectomized animals (mammals), but that in the intact animal the effect of treatment with TSH was thyroid stimulation.

Methods

Gravid goldfish (Carassius auratus) and gravid green sunfish (Lepomis cyanellus) were placed in 15-gallon glass aquariums supplied with running water and air. Human chorionic gonadotrophin (Sneed and Clemens 1959) and acetone-dried buffalo (Ictiobus sp.) pituitaries were used for the gonadotrophic hormones. A purified preparation of thyroid-stimulating hormone was obtained from a pharmaceutical supply house. The threshold dose (amount required to ovulate two of three injected fish) of chorionic gonadotrophin and fish pituitary was assumed to be 450 International Units (I.U.) and 1 milligram per pound of body weight, respectively, an assumption based on earlier experiments with goldfish. The data in table 1 (aquariums 9 and 11) also indicate these doses as a threshold level. The gonadotrophic materials were mixed with distilled water and injected intraperitoneally as described by Sneed and Clemens (1959, 1960). The levels of TSH were 1.3, 2.5, and 5 units per pound of body weight of the fish. The TSH was dissolved in distilled water and injected intraperitoneally.

Results of the Goldfish Experiments

In April 1960, mature female goldfish were recovered from an earthen pond, selected for similar physiological condition, and placed in aquariums. The goldfish received injections and responded as shown in table 1.

It is evident from the data obtained that thyroid-stimulating hormone alone, at levels as high as 10 units per pound, will not precipitate ovulation in the goldfish. However, ovulation occurred when 2.5 and 5.0 units of TSH per pound of body weight were combined with 225 units of chorionic gonadotrophin or 0.5 milligram of fish pituitary per pound of body weight. No ovulation occurred when 1.3 units of TSH was combined with either the chorionic gonadotrophin or fish pituitary. Therefore, thyroid-stimulating hormone in quantities of 2.5 units or more per pound of body weight does act to ovulate goldfish when injected with small doses of chorionic gonadotrophin or fish pituitary.

No ovulations occurred when either 225 I.U. of chorionic gonadotrophin, 0.5 milligram of fish pituitary, or 1 milligram of fish pituitary was administered alone. However, when the amount of chorionic gonadotrophin was 450 I.U. or the amount of fish pituitary was 2 milligrams per pound, ovulations were obtained. In these experiments, there is little evidence that results were greatly influenced by contamination of the TSH by gonadotrophins.

TABLE 1.--Demonstration of the effect of thyroid-stimulating hormone on goldfish with subthreshold levels of fish pituitary or human chorionic gonadotrophin

Aquarium	Number of fish injected	Injection[1]	Observations 18 hours after injection: Number ovulating	Observations 18 hours after injection: Number not ovulating
1	3	0.5 milligram pituitary + 1.3 units TSH	0	3
2	3	0.5 milligram pituitary + 2.5 units TSH	2	1
3	3	0.5 milligram pituitary + 5.0 units TSH	3	0
4	3	225 International Units APL + 1.3 units TSH	0	3
5	3	225 International Units APL + 2.5 units TSH	2	1
6	3	225 International Units APL + 5.0 units TSH	(2)	(2)
7	3	0.5 milligram pituitary	0	3
8	3	1 milligram pituitary	0	3
9	3	2 milligrams pituitary	3	0
10	3	225 International Units APL	0	3
11	3	450 International Units APL	2	1
12	3	900 International Units APL	3	0
13	3	1.3 units TSH[3]	0	3
14	3	2.5 units TSH[3]	0	3
15	3	5.0 units TSH[3]	0	3
16	3	0.5 milliliter distilled water	0	3

[1] Dosages based on body weight in pounds.

[2] Fish died because of mechanical failure in the water supply system.

[3] Fish reinjected 24 hours after the first injection, i.e., the fish received twice the amount in table. No ovulation observed after 4 days.

TABLE 2.--Demonstration of the effect of thyroid-stimulating hormone on green sunfish when injected with subthreshold levels of fish pituitary or chorionic gonadotrophin

Aquarium	Number of fish injected	Injection[1][2]	Observations 6 days after initial injections: Number ovulating	Observations 6 days after initial injections: Number not ovulating
1	3	0.5 milligram pituitary + 2.5 units TSH	[3]0	[3]2
2	3	0.5 milligram pituitary + 5.0 units TSH	2	1
3	3	1.0 milligram pituitary	[3]0	[3]2
4	3	2.0 milligram pituitary	0	[3][4]1
5	3	225 International Units APL + 2.5 units TSH	2	[4]0
6	3	225 International Units APL + 5.0 units TSH	3	0
7	3	450 International Units APL	0	3
8	3	900 International Units APL	0	3

[1] Dosages based on body weight in pounds.

[2] Fish were reinjected the third and fifth day after initial injections; i.e., they received three times the amount given in the table.

[3] One fish was a male.

[4] One fish died before termination of the experiment.

The fish ovulated when about half the normal threshold dose of gonadotrophin was combined with 2.5 units of TSH, which meant that TSH contributed half of the spawning principle. Therefore, twice this dose (5 units) should have produced ovulation if it were due to gonadotrophin alone. However, four times the amount (10 units) failed to cause ovulation.

Results of Green Sunfish Experiments

On April 29, 1960, gravid green sunfish were selected and put in aquariums. These fish received the injections and provided the results shown in table 2.

It is apparent from this experiment that the thyroid-stimulating hormone in some manner aids the action of either chorionic gonadotrophin or fish pituitary. Three injections of 225 I.U. of chorionic gonadotrophin combined with 2.5 and 5.0 units of TSH per pound of body weight resulted in the ovulation of all the fish. However, when four times that amount of chorionic gonadotrophin was used alone, no ovulations were obtained. Also, three injections of 0.5 milligram of fish pituitary combined with 5.0 units of TSH per pound of body weight resulted in ovulation of two of three fish; but when four times that amount of fish pituitary was used alone, no ovulations occurred. During the experiment a gravid green sunfish received one injection of 25 units of TSH. This fish (not included in table 2) ovulated a few eggs on the fourth day after injection. It was not a normal or complete ovulation, as the oviduct was inflamed and the fish appeared egg-bound.

The great difficulty experienced in the past and during this experiment in ovulating the green sunfish with hormones makes these data even more significant. Here is a possible method for spawning particularly refractory species such as the largemouth bass, which we have often failed to spawn with hormones. The TSH is expensive and not readily available on the market at present, but increased demand might change this situation. The additional cost to spawn a pound of female fish is about $3.

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LYMPHOCYSTIS AS A MORTALITY FACTOR IN A WALLEYE POPULATION

R. A. Ryder
Ontario Department of Lands and Forests
Sioux Lookout, Ontario, Canada

DURING A WALLEYE (Stizostedion v. vitreum) POPULATION STUDY initiated in the Nipigon River in the spring of 1955, it was noted that a large portion of the spawning fish were infected with lymphocystis. As the tagging operation had been expected to evaluate the extent of the walleye population, some concern was felt over the possibility of higher mortality occurring among those tagged fish which were so infected. The occurrence of lymphocystis was noticeably heavy only prior to, during, and shortly after the spring spawning runs.

To study the influence of infection upon rate of recapture, separate records were kept for healthy fish and for infected fish during part of the original study. In this study 1,504 walleyes tagged during the spawning runs of 1956 and 1957 were considered. All the fish were marked with streamer tags as outlined by Joeris (1953). The fish were selected as randomly as possible with dip net and seine, no effort being made to obtain either healthy or diseased fish. Scale samples were not taken from the tagged fish, nor were they mutilated in any obvious manner which might have increased their susceptibility to contamination with lymphocystis. Tag returns were made by anglers, commercial fishermen using gill nets and pound nets, operators of a lamprey weir on the Jackfish River, and Department of Lands and Forests personnel using experimental gill netting.

Lymphocystis is described by Watson (1954) as a disease of virus origin in which the infected cells undergo hypertrophy. It has been found on a variety of marine and fresh-water fishes, of which the walleye appears to be especially susceptible. Walker (1957) noted that in the United States the walleye, which is the only species of fish having a consistent record of occurrence of lymphocystis, is infected at rates ranging generally from a fraction of 1 percent to 5 percent.

Little is known of the rate of mortality that results directly from lymphocystis infection, though Watson (1954) stated that high mortalities had not been reported. Olson (1958) established that the handling and marking of walleyes resulted in increased occurrence of lymphocystis. He observed that fin-clipped walleyes in the spawning runs had an infestation rate of 3 to 1 when compared with unmarked fish. Olson did not believe that a differential mortality occurred, and noted that angler returns indicated that the disease had nearly disappeared in the summer.

Incidence of Lymphocystis

Table 1 shows the frequency of occurrence of lymphocystis in walleyes during the 1956 and 1957 spawning runs in the Nipigon River. In this table the tag returns for the 2 years immediately after tagging are given for infected and sound fish. The data are subject to the variables discussed by Ryder in his paper on the influence of the anesthetic employed (1960). In 1956, urethane was used as the anesthetic for all but the last 56 walleyes

TABLE 1.--Tag returns from lymphocystis-infected and sound walleyes for the two years after tagging

Date tagged	Sound fish tagged		Infected fish tagged		Tag returns from sound fish		Tag returns from infected fish	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
1956	752	75.2	248	24.8	148	19.7	55	22.2
1957	357	70.8	147	29.2	108	30.5	52	35.4

tagged, and for these ether was used. In 1957, all the walleyes tagged were anesthetized with tricaine methanesulfonate (M. S. 222). Consequently, the type of anesthetic employed may influence the results in a comparison of 1956 and 1957, but should not affect results in a comparison of the rates of tag returns for infected and sound fish in either of these years. Table 1 shows that in both 1956 and 1957 the proportion of tag returns from infected walleyes was noticeably higher. If lymphocystis did not increase the mortality rate of walleyes, it could be expected that the rate of tag returns for infected and sound fish would be approximately the same within the limitations of the experiment. A higher rate of tag returns from infected fish could be explained by either of two conditions, or by a combination of them, namely: (a) The size of the sample of tag returns from the infected fish was inadequate; or (b) diseased fish were more vulnerable to capture, particularly by gill nets, because of the "warty" protuberances on their bodies and fins. This second condition appeared to be applicable in the Nipigon Bay commercial fishery, which captured large numbers of lymphocystis-infected fish for a 2- or 3-week period immediately after spawning in the Nipigon River. During the remainder of the year the incidence of lymphocystis decreased until it was almost nonexistent in July. The active fall gill-net fishery in Nipigon Bay did not report lymphocystis-infected walleyes.

Table 2 presents the temporal distribution of infected walleyes in the spawning period of 1956. There was an observable increase in the incidence of lymphocystis during the 10 successive days on which the fish were tagged. It is possible that the large number of fish inhabiting the shallow riffle area of the river lost some of their protective mucous covering while swimming close to the boulder substrate. The congestion of the walleyes in the riffle area at this time of year would be likely to facilitate the spread of the disease from infected to sound fish. The incidence of the disease would possibly increase during spawning, as the lymphocystis cells drop off infected fish and contaminate healthy fish that have lost some of their protective mucous covering. Nigrelli (1954) described the sloughing of lymphocystis cells, which are not cemented to neighboring tissue.

In the present study the tags of all walleyes that were returned, and the fish made available for examination, were found not to be infected when the returns

TABLE 2.--Progressive increase in the incidence of lymphocystis-infected walleyes in the spring spawning run, Nipigon River, 1956, from 1,000 fish sampled

Date	Sound fish	Infected fish	Percentage infected
Apr. 30	47	10	17.5
May 1	86	21	19.6
May 2	84	22	20.8
May 3	102	30	22.7
May 4	73	29	28.4
May 5	83	23	21.7
May 6	83	27	24.5
May 7	73	34	31.8
May 8	82	35	29.9
May 9	39	17	30.4

were made between June 30 and November 30. Tagged fish taken during April or May in subsequent spawning periods, 1 or 2 years after tagging, and those taken in the spring gill-net fishery in Nipigon Bay were found to have a high incidence of infection. The period of observation for recaptures included April to November each year. There were no tag returns from the end of November to the following March, although many walleyes from the Nipigon River population were examined during this period and found to be free of lymphocystis infection.

Hile (1954) observed that lymphocystis infection in Saginaw Bay walleyes was highest in the fall of 1942 and occurred somewhat less frequently in the spring of 1943. He recorded an incidence of infection of 6.6 percent for May 1943. He also reported that lymphocystis-infected walleyes in Saginaw Bay weighed slightly less than sound fish for each 0.25-inch length grouping. Nigrelli (1954) stated that in temperate fresh-water fishes the occurrence of lymphocystis is first noticeable in the spring, reaches a peak in summer, and gradually disappears in the fall and winter. Witt (1957) noted that the literature includes conflicting reports about the time of year at which the incidence of lymphocystis reaches a maximum. Recording the incidence of lymphocystis in a white crappie (Pomoxis annularis) population, Witt found that the rate of infection rose in the spring of 1950, reached a peak in July, declined gradually in August and then fell rapidly until it reached a low in October, rose sharply in November, and thereafter tapered off until it reached a minimum in April 1951; after April, the rate rose again until in June 1951 it was as high as it had been in June 1950.

Summary and Conclusions

1. Tag returns were greater for the lymphocystis-infected walleyes than for sound fish. This could be attributed to greater vulnerability to gill nets because of the "warty" protuberances on infected fish, or possibly to an inadequate sample of tag returns from infected fish.

2. Incidence of lymphocystis-infected walleyes increased during the spawning period from 17.5 percent at the start of tagging to 30.4 percent at its termination 10 days later. Lymphocystis may have resulted from the removal of the mucous coating of the walleyes when in the shallow riffle areas, an increased rate of contamination being due to the congestion of the fish on the spawning grounds.

3. Lymphocystis is at a high level only during and immediately after the spring spawning period of the Nipigon River walleyes. Tagged infected fish showed no traces of the disease in summer and fall. Fish of the same population examined in the winter had no traces of the disease.

4. Mortality of lymphocystis-infected walleyes in the Nipigon River is probably negligible and will not influence population estimates based on tag returns. A slight downward adjustment may be necessary to compensate for greater vulnerability of infected fish to capture by gill nets.

Acknowledgments

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Mr. K. H. Loftus reviewed the manuscript.

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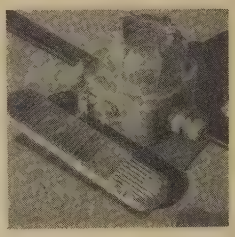
ELECTROFISHING SAFETY OR "SURPRISE" SWITCH FOR USE IN SMALL BOATS

Electrofishing in the Florida Everglades is done from an airboat where the individual dip-netting the shocked fish has to stand on a comparatively small deck. As there is nc railing or other protection from falling overboard, a heavy-duty foot switch has been obtained to protect the collector if he should slip or lose his balance. While the individual is standing on the switch pedal, the current is flowing to the electrodes; but if his foot is removed for any reason, the current is automatically shut off.

In addition to the safety value of this switch, there is the "surprise" aspect. It has been observed that fish swim ahead of an electrical field, or leave their hiding place before the collector is close enough to capture them, if the electrical current is passing continuously to the electrodes. However, efficiency of collecting increases if the current is not turned on until the electrodes are situated in a likely looking spot for the fish--or if the current is just turned on for short intervals as the boat moves through the collecting area. The collector can turn the current on or off at his discretion by simply shifting his foot, thus leaving both his hands free to use the dip net.



The switch is easily moved from the airboat to an outboard-motor boat when the collecting is done in water that is free of vegetation. Waterproof and sturdy, the switch has an 8-hour rating of 25 amperes. It is being used in work supported through the Federal Aid to Fish Restoration Program, Dingell-Johnson Project F-8-R, Florida.

--JAMES P. CLUGSTON, Game and Fresh Water Fish Commission, Fort Lauderdale, Florida.

STANDARDS FOR REPORTING FISH TOXICITY TESTS

Oliver B. Cope
Bureau of Sport Fisheries and Wildlife
Fish-Pesticide Research Laboratory, Denver 25, Colorado

THE GROWING IMPETUS OF STUDIES ON FISH AND PESTICIDES focuses attention on the need for standardized reporting procedures. Good methods have been developed for laboratory and field procedures in testing programs and in statistical features of assay experiments; and improvements are being made on methods of collecting and preserving fish, invertebrates, and other materials exposed to economic poisons. On the other hand, the reporting of toxicity data in a complete manner has lagged behind, and today's literature is little improved over yesterday's with regard to completeness and susceptibility to interpretation.

One of the important uses of toxicity information from field and laboratory experimentation is to furnish comparisons with similar work performed by other workers. The multiplicity of factors that can affect the action of a toxicant in any situation renders it mandatory for the writer to characterize the conditions under which the experiment is conducted. A large share of the pesticide reports appearing in today's literature cannot be compared with other results because of failure to specify the essential particulars surrounding the work. On the other hand, some excellent papers appear in the literature; a few examples of completeness in reporting are listed at the end of this paper.

Three types of information must be kept in mind for reports on toxicity to fish. One of these concerns the fish themselves; the second pertains to the methods used in the experiment; the third has to do with the environment. Methods of communicating data on these factors are discussed in the following treatment.

The Fish

The identity of fish used in toxicity experiments can be of great importance in judging their resistance to various toxicants. Accordingly, the author should never fail to designate the species and subspecies; if the race or stock is known, i.e., the Manchester strain of rainbow trout, it should be mentioned. The source of the fish, wild or hatchery, is useful information for the reader. The size of fish, in length or weight, is essential, as is some measure of the condition, expressed either as condition factor or as an observation, vigor, presence of disease or parasites, sex, maturity, and any other measurements, such as haematological determinations from a sample of the fish stock, which may help to establish the basic fitness of the test animals.

The Environment

The report on every toxicity test should furnish an accurate and complete description of the physical, chemical, and biological setting in which the test was laid. Only by having an understanding of this setting can the reader fairly compare the responses of the fish with results from other treatments.

The volume of water used in aquarium tests can have a bearing on toxicity and should be reported, along with the number

of fish per volume. For field tests, the depth of water and its movement should be explained. In the area of physical characteristics of the water medium, temperatures prevailing at test time are important. The chemical constitution of the water has such an important influence on toxicity that the author must not fail to characterize it. If detailed measurements of the pH, alkalinity, and total dissolved solids are not available, at least an expression of hardness should be put forth. Any unusual features of water chemistry in laboratory or field media should be included in the description of the environment.

Prime features of the environment in experiments on poisons and fish are the characteristics and mode of presentation of the toxicant itself. It is essential to identify the chemical tested, its purity (technical grade, commercial, etc.), its formulation, its concentration, and the length of time during which the fish were exposed. It must be made clear whether the concentration is in terms of active ingredient or otherwise. In field treatments in which poisons are broadcast on an areal basis, it is most helpful to the reader if the report shows a theoretical conversion to a weight-volume (such as milligrams per liter) basis, based on the depth of the water, a knowledge of the distribution of the chemical, and the rate of flow in moving waters.

Another important feature of the environment in field toxicity tests is the biota. The dominant flora and fauna in lakes and streams should be described. Other items worthy of reporting for some situations are the physical and chemical natures of lake and stream bottom materials, and the light conditions prevailing during the exposure period.

Methods

An account of the methods followed in the conduct of the tests is a vital part of the reporting. A bare minimum in this would be an explanation of the general plan of the experiment, from the pretesting holding phase to the summary of the responses of the fish. A more complete description would include the conditions under which holding was performed, time of holding, time of fasting, routine disease and parasite prophylaxis and therapy, and pretreatment reactions. Reference to standard methods is a useful approach.

The method of applying the toxicant to the water is important. For field tests, the report should specify the use of airplane or ground spray methods, including characteristics of the spray patterns. For laboratory tests, it should be made clear that the stock solution was dripped at a certain rate, placed into solution before fish were added, or subjected to other methods of administration.

The statistical plan of the test should be made clear, and the statistical treatment of the data identified. Whether dosage-mortality or time-mortality relations are the objective of the tests, the results should be expressed in completely usable form for the reader. Probit analysis is an excellent tool for handling these problems; the curves are expressed as straight lines and simplify comparison. Whether probits or other statistical tools are used, it is essential to provide a measure of dispersion about the curve or the point of comparison so that the soundness of the experiment can be evaluated. The curves can be presented on graphs and the dispersion indicated; or, when curves are not desired, tables can show the TL_m, LD_{50}, LD_{90}, or any other point on the curves as numerical values, followed by the standard deviation and the slope of the straight-line curve when a logarithmic basis is used. Confidence limits should be presented.

Summary

It is essential that authors prepare their reports on tests involving the toxicity of substances to fish in such a way as to aid the reader in gaining a full appreciation of the probable significance of the results. Completeness is desired in characterizing the test animals, in explaining methods used, and in identifying

components of the environment. These requirements apply to both field and laboratory experiments. Although the number of points covered in this discussion may seem excessive, carefully prepared descriptions of circumstances surrounding toxicity tests can be included without throwing a report out of balance, and the value to the reader can be much improved.

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AN ATTEMPT TO USE SODIUM SULFITE TO FACILITATE THE RECOVERY OF POND-REARED WALLEYES

The use of sodium sulfite to assist in fish removal as reported in 1956 by Westman and Hunter (THE PROGRESSIVE FISH-CULTURIST, 18 (3): 126-130) was attempted at the Palmer State Hatchery in August 1960.

At this hatchery a rectangular 0.33-acre pond containing 0.66 acre-foot was treated with 100 p.p.m. (200 pounds) of sodium sulfite. Observations during the following 5 hours failed to reveal any of the distressed fish activity reported by Westman and Hunter.

Dissolved-oxygen determinations that were taken 20 hours after the initial application showed concentrations ranging from 2.5 to 3.1 p.p.m. at a water temperature of 76° F. In the original experiment, Westman and Hunter suggested that oxygen concentrations should be reduced to 1.0 p.p.m. in order to bring the fish to the surface. Therefore, another 100 pounds of sodium sulfite was added. This brought the total concentration applied to the pond within a 22-hour period to 150 p.p.m. Again there was no indication of any reaction from fish that were known to be in the pond. Six hours after this second application of the sodium sulfite, another series of oxygen samples showed concentrations of 1.9 to 3.6 p.p.m.

A short time after the second application of this material, 125 fingerling walleyes were seined from the pond and placed in a holding tank. These fish appeared to be in good condition, and they experienced no undue mortality in the subsequent weeks.

Eight days after these applications of sodium sulfite, the pond was drained and 75 more walleyes were recovered from it. Apparently the application of 150 p.p.m. of sodium sulfite during a 22-hour period caused no fish mortality, but neither was it successful in bringing the walleyes to the surface for easy capture. Undoubtedly an insufficient concentration resulted because of the rapid breakdown of this material between applications.

--FRANK GRICE, Division of Fish and Game, Westboro, Massachusetts.

AN IMPROVED TRAP FOR THE CAPTURE AND SAFE RETENTION OF SALMON SMOLTS

Donovan R. Craddock
Bureau of Commercial Fisheries,
Seattle, Washington

ONE OF THE FUNDAMENTAL PROBLEMS IN MANY FISHERY PROGRAMS is the development of equipment and techniques to obtain the fish samples necessary for the study. Research to determine natural survival and production of salmon depends on assessing the size and composition of downstream migrations. The mechanics of capturing the required smolt samples can be a difficult problem where the river is fairly large and funds and manpower are limited.

Weirs are costly to install and may be difficult and expensive to maintain. Fyke nets, on the other hand, have proved to be effective in capturing migrating fish in flowing waters. They are not expensive to obtain, but require considerable manpower to operate because almost constant attention is necessary if the captured fish are to be retained alive. This latter fact precludes use of the nets where large numbers of migrants are required for a mark-and-recovery program with a small staff. Modifications have improved fyke nets for small-scale sampling in small streams (Craddock 1959), but these modified nets are not adequate for the capture and live retention of great numbers of fingerlings.

The trap described here can be used to advantage in many projects where it is important to capture large numbers of downstream migrants and maintain them alive over extended periods of time. It is especially adaptable to projects involving moderately large rivers and limited manpower. It is also inexpensive; a complete trap costs approximately $200.

This trap is essentially a winged fyke net connected to a floating livebox by a flexible hose. The construction details of the trap are shown in figure 1.

The pattern used to cut the netting is shown in figure 2. It is very important that the netting be cut and joined to form a tight cone and not belly out in the current. All seams in the net are reinforced with 1-inch nylon tape, and the cod end is completed with a collar of 4-inch nylon tape. The hose couplers are connected to the hose and net by adjustable metal bands. The terminal coupler is bolted to the opening in the livebox.

The successful operation of this trap depends on an adequate current to capture the fish and carry them into the livebox. If the velocity is too low, the fish will avoid the trap; if the velocity is too great, the operation of the trap will become difficult. The trap should operate well in velocities ranging from 2 to 4 feet per second.

The net can be operated in water more than 4 feet deep; but when depths exceed 4 feet, sufficient flotation should be secured to the top of the large frame to keep it on the surface. The angle of the wings may be adjusted, depending on the current, so that the greatest area is covered without causing the wings to belly out.

Adequate anchoring is very important to the successful fishing of these traps. A cable secured across the river offers an excellent solution to this problem; pulleys attached to the bridle will allow the trap to be moved along the cable from

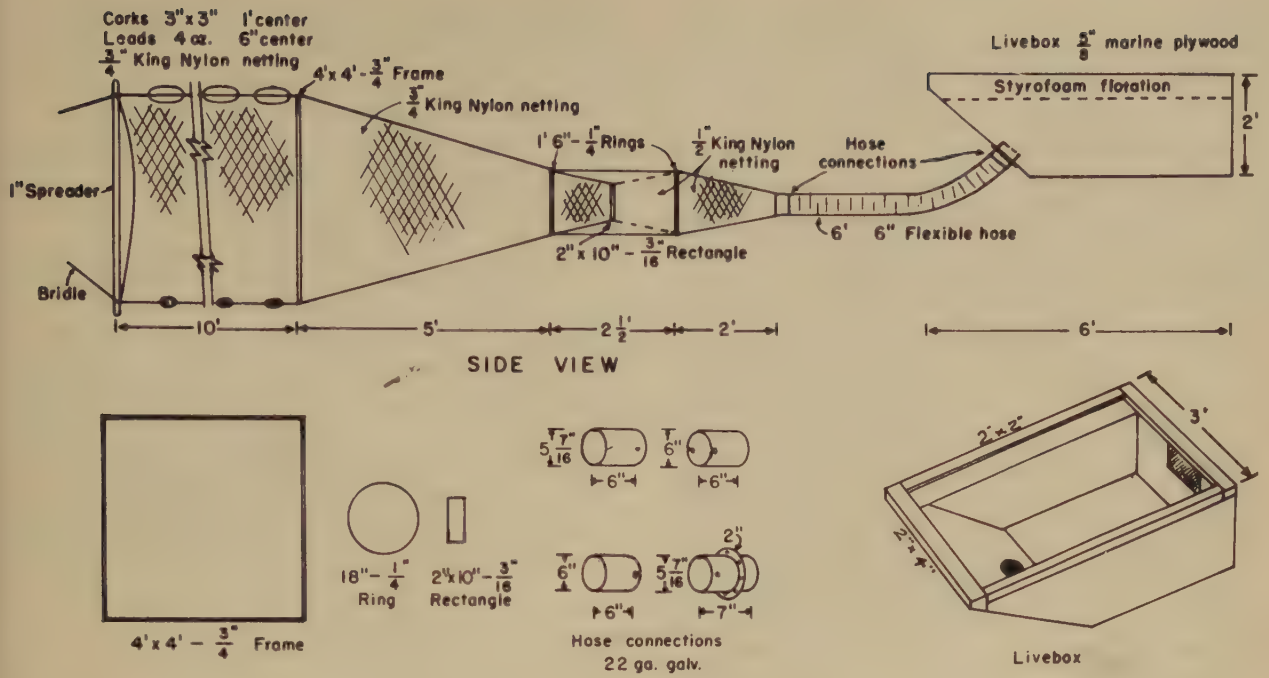

FIGURE 1.--An improved live trap for the capture of salmon smolts. (Not indicated: the flexible hose is high-tensile steel wire covered with neoprene-impregnated canvas; the styrofoam is in 2- by 5-inch strips.)

one location to another. The trap is anchored by the two bridle lines; the livebox is secured by one line just tight enough to take some of the strain off the net.

When the trap is operating properly, the top of the main frame is at the surface of the water and the net extends downstream on a horizontal plane. The hose makes a gentle arc upward to the livebox, which floats on an even keel with about 5 inches of freeboard. A good flow enters from the hose and leaves through the screened opening at the back of the livebox. One of the most important considerations in the

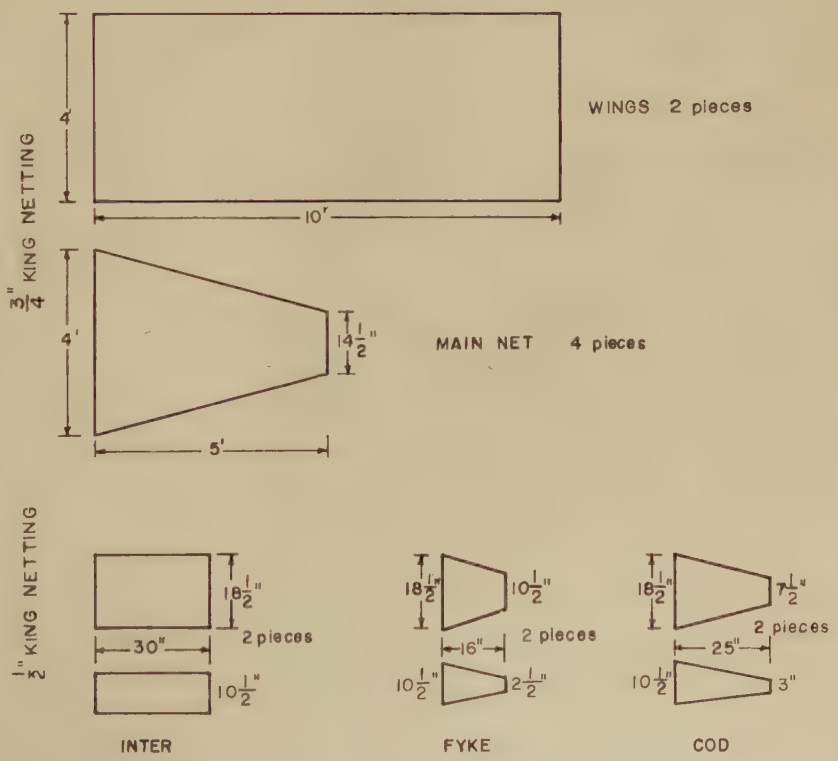

FIGURE 2.--Pattern used to cut nylon netting.

maintenance of the net is to keep it free of debris which might damage the netting or clog the throat and cause mortalities among the captured fish.

Three of these traps were employed with good success during the spring of 1960 in capturing blueback salmon in the Wenatchee River, Washington. At the trapping site the river was approximately 240 feet wide and had a flow of 1,100 to 5,110 cubic feet per second and an estimated velocity of 1.25 to 3.50 feet per second. During the 6-week migrant season these three traps caught more than 37,000 fingerlings and individual traps held as many as 3,000 fish in good condition for periods as long as 10 hours. The only mortalities of consequence occurred when the throat of the net became choked with debris and the fish were kept from passing into the livebox.

In testing for size selectivity of the nets, large (-85 millimeters) and small (-80 millimeters) blueback smolts of the same age group were marked and released. Of approximately 2,000 of each size released, 4 percent of the large and 8 percent of the small were recovered in the nets. Statistical tests verified that the nets were selective for the smaller fish. The above percentages will probably change with changing velocities, and tests should be made to determine selectivity in each new situation.

The main advantages of this trap are: (1) it has the fish-catching ability of a common fyke net, (2) it holds fish alive in good condition for extended periods of time, (3) it requires relatively little attention, and (4) it is economical to construct and operate. The limitations of the trap are: (1) it may be selective to size of fish, and (2) its efficiency may change with changing water flows.

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CARBON DIOXIDE CYLINDER ADAPTED FOR APPLICATION OF LIQUID CHEMICALS

Portability and one-man operation are desirable features in equipment for applying liquid chemicals in fish management. I should like to pass on to other fishery workers the simple and economical equipment that I have devised for the purpose.

I use a 10-pound carbon dioxide cylinder with a connecting pressure regulator. An ordinary, easily acquired replacement hose for a hand tire pump carries the carbon dioxide from the regulator to the drum of chemical material.

The carbon dioxide hose connection on the drum is made by drilling and tapping a hole in the large plug to accept a short, threaded truck-tire valve stem. By this method, any drum having a standard-size bunghole can be used with the pressure bottle. A 0.75-inch faucet is screwed into the new small hole to let the pressurized liquid out, and a 0.5-inch perforated garden hose is screwed on the faucet to apply the chemical. If a clear plastic hose is used, there is no problem in determining when the drum is emptied.

The 15-gallon drum that I use can be emptied in about 6.5 minutes using 10 pounds' pressure. A 30-gallon drum can be emptied in about 12 minutes. A full cylinder of carbon dioxide will take care of 15 of the 15-gallon drums.

The complete outfit costs about $50, though this may be reduced by renting or borrowing the cylinder. Cost of filling the cylinder is about $3.

I have applied liquid rotenone preparations in water 50 feet deep as easily as in shallow water.

--E. W. COLEMAN, Game and Fish Commission, Cleveland, Mississippi.

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WAIKIKI AQUARIUM
2777 KALAKAUA AVENUE
128 C HONOLULU T H

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